CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	761125
Supporting document/s:	• SD 4 (new BLA, submitted 2/07/2019) • SD 8 (Response to Information Request, submitted 4/30/2019) • SD 26 (Response to Information Request, submitted 8/12/2019)
Applicant's letter date:	February 7, 2019
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Product:	Beovu™ (Brolucizumab)
Indication:	Treatment of neovascular age-related macular degeneration (AMD)
Applicant:	Novartis Pharmaceuticals Corporation East Hanover, New Jersey, 0736-1080
Review Division:	Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP), CDER, HFD-590
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1 Executive Summary

1.1 Introduction

- In accordance with section 351 of the Public Health Service Act and 21 CFR 601.2, Novartis Pharmaceuticals Corporation (Novartis) submitted an original Biologics License Application (BLA) for brolucizumab injection for the treatment of neovascular (wet) age-related macular degeneration (nAMD).
- Brolucizumab (RTH258, ESBA1008, AL-86810) is a 252 amino acid humanized single-chain antibody fragment (scFv) which inhibits vascular endothelial growth factor A (VEGF-A) binding to its receptors VEGF receptor 1 (VEGFR1) and VEGFR2. It is produced in *Escherichia coli* by recombinant expression technology using Good Manufacturing Practices (GMP).
- The Applicant has proposed a proprietary name (trade name) of Beovu™.
- Clinical trials to support the AMD indication were conducted under IND 112023.
- Novartis explained (BLA module 2.2 Introduction to Summary):
    - Brolucizumab binds to and inhibits the biological activity of human endothelial growth factor A (VEGF-A) by preventing it from activating its receptors, VEGFR1 and VEGFR2, on the surface of endothelial cells, thereby reducing endothelial cell proliferation, vascular permeability/leakage, and new blood vessel formation (neovascularization).
    - Brolucizumab has been shown to bind the three main isoforms of human VEGF-A: $VEGF_{110}$, $VEGF_{121}$, and $VEGF_{165}$.
    - Inhibition of the VEGF pathway has been shown to inhibit the growth of neovascular lesions and resolve retinal edema in patients with nAMD resulting in improved and/or maintained visual function.
    - The smaller size of the scFv allows for a higher molar dose (i.e. higher molar concentration) compared to other approved intravitreal anti-VEGF products.
    - “Brolucizumab represents an important therapeutic advance for patients with nAMD. In two adequate and well-controlled trials, brolucizumab demonstrated non-inferior visual acuity gains and superior anatomical outcomes over current standard of care (aflibercept) at Week 48. These effects were achieved with a majority of patients treated with brolucizumab 6 mg every 12 weeks. Efficacy was maintained for 2 years. The overall safety profile of brolucizumab 6 mg administered over 2 years was comparable to aflibercept and consistent with that reported in Phase III trials of other marketed anti-VEGF treatments. These data clearly demonstrate a positive benefit to risk profile for the use of brolucizumab 6mg for the treatment of patients with nAMD.”
- The nonclinical Pharmacology/Toxicology (P/T) review of the BLA is complete, and P/T recommends approval. From a P/T perspective, no safety or regulatory issues were identified that would preclude approval.

- The submission is available internally via the electronic document room (EDR): \\cdsesub1\evsprod\bla761125\

1.2 Brief Discussion of Nonclinical Findings

- Brolucizumab is a single-chain, 252 amino acid, scFv that binds to VEGF-A, and inhibits the binding of VEGF-A to VEGFR1 and VEGFR2.

Pharmacology

- The *in vitro* primary pharmacodynamic (PD) studies to demonstrate this anti-VEGF mechanism of action are robust, and generally consistent.
- Based on comparison of the *in vitro* results to the clinical pharmacokinetic (Clinical Pharmacology) results, P/T predicts that brolucizumab will exhibit systemic activity in animals and patients following intravitreal (ivt) dosing.
- *In vivo* PD studies in the rat and mouse demonstrated proof-of-concept for ivt brolucizumab to treat the retinal vasculature.

Species selection:

- The Applicant assumed that the cynomolgus monkey is a pharmacologically relevant model for brolucizumab because the amino acid sequences of human and cynomolgus monkey $VEGF_{165}$ are identical. P/T concurs with this approach.
- Binding experiments testing single isoforms of VEGF per species showed that brolucizumab binds human, rat, mouse, dog, pig, and cat VEGF with comparable potency. No functional assays were conducted to verify cross-species activity. The rat model as well as other common laboratory species may be pharmacologically relevant models for brolucizumab testing. Brolucizumab did not bind a short rabbit VEGF isoform (i.e. rabbit may not be a relevant model, or may be only partially relevant).
- The Applicant proposed to use the monkey as the sole animal species used for intravitreal (ivt) toxicology testing, and DTOP concurred. Based on anatomy and physiology, the monkey is considered the model most predictive for the effects of intraocular dosing on the retina.
- Nonclinical testing to address developmental and reproductive toxicity (DART) has not yet been submitted to DTOP.

Pharmacokinetics (PK)

- Four nonclinical PK studies were conducted with brolucizumab.
  - Following a single intravenous (iv) injection, brolucizumab clearance was rapid. The initial elimination half-life ($t_{1/2}$) was < 30 minutes. The terminal $t_{1/2}$ was 1.5 hours.
  - Following single intravitreal (ivt) injections into the eyes of monkeys or rabbits, ocular distribution was consistent with expected distribution for proteins. Brolucizumab distributed from the vitreous into the retina, choroid, aqueous humor, and blood.
  - Anti-drug antibodies (ADA) to brolucizumab were detected occasionally in animals prior to the start of dosing (i.e. animals already had antibody

cross-reactivity to brolucizumab). Generally, new ADA formation was low, and did not affect PK/TK in the animal models.

- Systemic TK and ADA were also measured in each of the GLP toxicology studies.
- Systemic exposure (i.e. serum) was consistently detected after ivt dosing.

Toxicology

- Early nonclinical studies tested a pre-GMP lot of brolucizumab; the ocular toxicity observed are not relevant to the GMP commercial lot. Review of those studies is documented below (for completeness of record), and to highlight potential safety questions (i.e. affected endpoints were checked carefully for the GLP toxicology studies).
- The Applicant submitted three Good Laboratory Practices (GLP)-compliant toxicology studies to the BLA. Each used intravitreal (ivt) injection to deliver brolucizumab unilaterally (single-eye) of cynomolgus monkeys. Taken together, the results supported the safety of clinical trials with brolucizumab, and support the safety of the BLA.
    - The IND-enabling toxicity study (report # TDOC-0012707) administered brolucizumab at 3 week intervals, with 3 doses total (Q3Wx3). The doses tested were 0, 0.5, 1, 3, or 6 mg/eye of brolucizumab.
        - Ocular inflammation was observed at 0, 0.5, 1, and 3 mg/eye (but not 6 mg/kg), and was not considered intolerable or dose-limiting. DTOP concluded that the results supported the safety of the first-in-human clinical trial (C-10-083) for treatment of patients with AMD.
    - The pivotal study supporting the BLA is the 6-month ocular toxicity study (report # TDOC-0016684). Cynomolgus monkeys were dosed ivt once every 4 weeks, for a total of doses (Q4Wx6). The dose levels tested were 0, 1, 3 or 6 mg/eye. Two different formulations were compared.
        - The high-dose of 6 mg/eye was established as the ocular no observed adverse effect level (NOAEL). The battery of ocular endpoints was adequate to detect potential toxicity, and no treatment-related ocular effects were observed.
        - The high-dose of 6 mg/eye was established as the systemic NOAEL. Treatment with ≥ 3 mg/eye increased spleen weight. The increases were not considered adverse and no histopathology or hematology correlate was observed.
        - The results of this study support chronic dosing of patients with brolucizumab.
    - A GLP 3-month study (report # TDOC-0017689) with limited safety endpoints was conducted, to support a change in manufacturing. Cynomolgus monkeys were dosed ivt with 0 or 6 mg/eye once every 4 weeks, for 3 total doses (Q4Wx3).
        - Ocular toxicity was not observed.
        - Treatment caused a non-adverse increase in spleen weight (i.e. consistent with report # TDOC-0016684).

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, P/T recommends approval of BLA 761125.

1.3.3 Labeling

The Applicant proposed labeling in the original BLA submission (2/07/2019).[1]

Applicant's proposed language (2/07/2019)	P/T recommended language:
INDICATIONS AND USAGE TRADENAME is indicated for the treatment of Neovascular (Wet) Age-Related Macular Degeneration (AMD) (1)	**INDICATIONS AND USAGE** **BEOVU is a vascular endothelial growth factor (VEGF) inhibitor** indicated for the treatment of Neovascular (Wet) Age-Related Macular Degeneration (AMD) (1)
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of TRADENAME administration in pregnant women, (b) (4) [redacted] (b) (4) [redacted] based on the anti-VEGF mechanism of action [see Clinical Pharmacology (12.1)], (b) (4) [redacted] the potential risks to the fetus. The background risk of major birth defects and miscarriage for the indicated	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of TRADENAME administration in pregnant women (b) (4) [redacted] **Based on the anti-VEGF mechanism of action for brolucizumab [see Clinical Pharmacology (12.1)], treatment with BEOVU may pose a risk to human embryofetal development. BEOVU should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.** **All pregnancies have a background risk of birth defect, loss, and other adverse outcomes.** The background

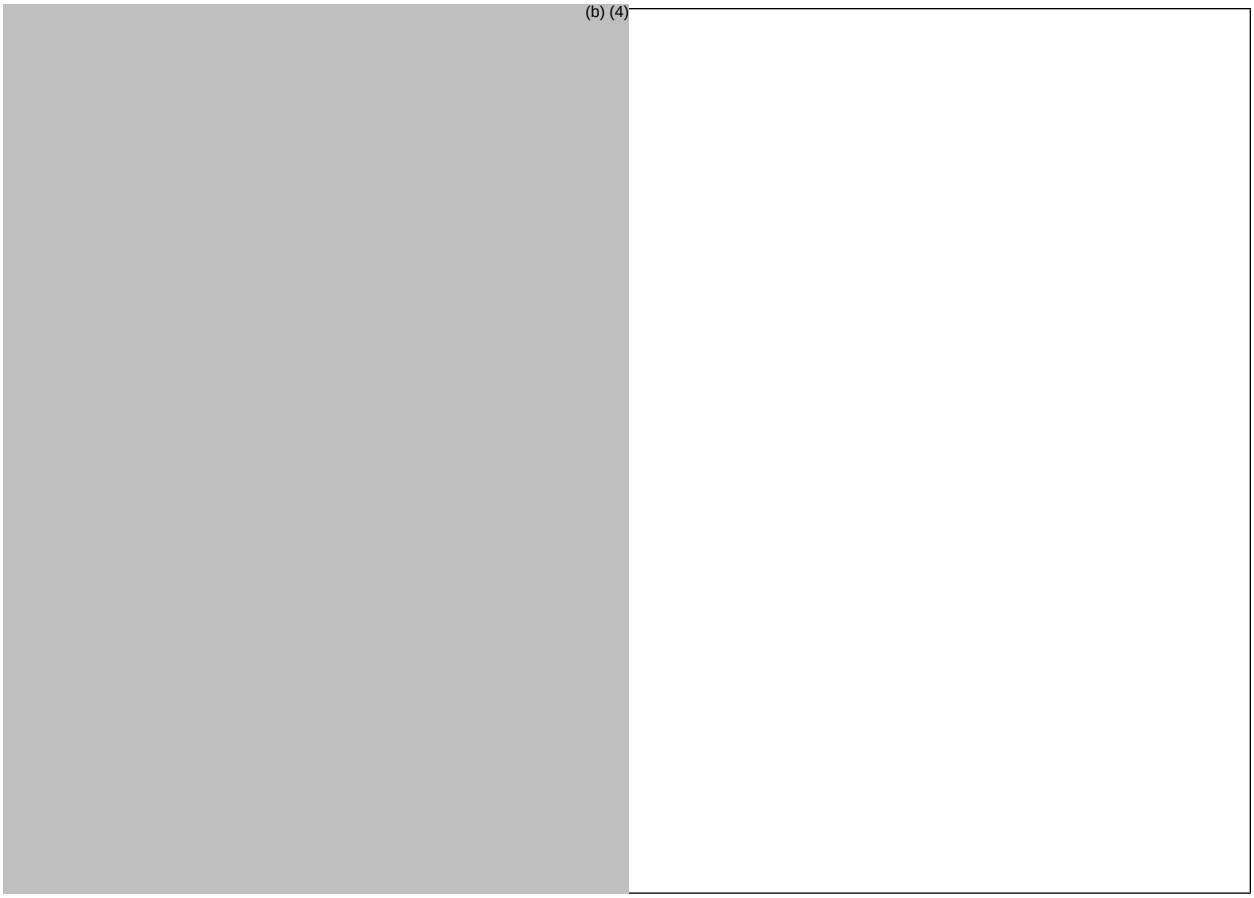
[1] BLA module 1.14.1.2 Annotated Draft Labeling Text accessed via: \\cdsesub1\evsprod\bla761125\0001\m1\us\annotated.pdf

population is unknown; (b) (4) in the U.S. general population, the estimated background risk of major birth defects is 2%-4% and of miscarriage is 15%-20% of clinically recognized pregnancies.	risk of major birth defects and miscarriage for the indicated population is unknown. (b) (4) In the U.S. general population, the estimated background risk of major birth defects is 2%-4% and of miscarriage is 15%-20% of clinically recognized pregnancies.
Data (b) (4) VEGF inhibition has been shown to affect follicular development, corpus luteum function, and fertility. (b) (4)	Data **Brolucizumab is a VEGF inhibitor; it distributes into systemic circulation after intravitreal injection.** (b) (4) **Angiogenesis is important for critical aspects of female reproduction, embryofetal development, and postnatal development. VEGF inhibition has been shown to cause malformations, embryofetal resorption, and decreased fetal weight**. VEGF inhibition has **also** been shown to affect follicular development, corpus luteum function, and fertility. (b) (4)
8.2 Lactation Risk Summary There is no information regarding the presence of brolucizumab in human milk, the effects of the drug on the breastfed infant, or the effects of the drug on milk production/excretion. Because many drugs are transferred in human milk, and because of the potential for absorption and adverse reactions in the breastfed child, breastfeeding is not recommended during treatment and for at least one month after the last dose when stopping treatment with TRADENAME.	[no changes]

8.3 Females and Males of Reproductive Potential Contraception *Females* [redacted] of reproductive potential should use effective contraception (methods that result in less than 1% pregnancy rates) during treatment with TRADENAME and for at least one month after the last dose when stopping treatment with TRADENAME	**8.3 Females and Males of Reproductive Potential** Contraception *Females* [redacted] of reproductive potential should use **highly** effective contraception (methods that result in less than 1% pregnancy rates) during treatment with TRADENAME and for at least one month after the last dose when stopping treatment with TRADENAME**.** **Infertility** **No studies on the effects of brolucizumab on fertility have been conducted and it is not known whether brolucizumab can affect reproductive capacity. Based on its anti-VEGF mechanism of action, treatment with BEOVU may pose a risk to reproductive capacity.**
12.1 Mechanism of Action [redacted] Brolucizumab binds [redacted] three major isoforms of VEGF-A (e.g., VEGF110, VEGF121, and VEGF165) with receptors VEGFR-1 and VEGFR-2. By inhibiting VEGF-A, brolucizumab [redacted] suppresses endothelial cell proliferation, [redacted] neovascularization and [redacted] vascular permeability.	**12.1 Mechanism of Action** **Brolucizumab is a VEGF inhibitor.** [redacted] Brolucizumab binds [redacted] the three major **human** isoforms of VEGF-A (~~e.g.,~~ VEGF110, VEGF121, and VEGF165), **thereby preventing interaction** with receptors VEGFR-1 and VEGFR-2. By inhibiting VEGF-A, brolucizumab [redacted] suppresses endothelial cell proliferation, [redacted] neovascularization and [redacted] vascular permeability.
12.3 Pharmacokinetics [redacted]	[no changes proposed; language is captured in this review as context for the P/T results]

(b) (4)	
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** No studies have been conducted on the carcinogenic or mutagenic potential of TRADENAME.	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** No studies have been conducted on the carcinogenic or mutagenic potential of BEOVU. **Based on the anti-VEGF mechanism of action, treatment with TRADENAME may pose a risk to reproductive capacity [see Females and Males of Reproductive Potential (8.3)**
(b) (4)	(b) (4)

(b) (4)



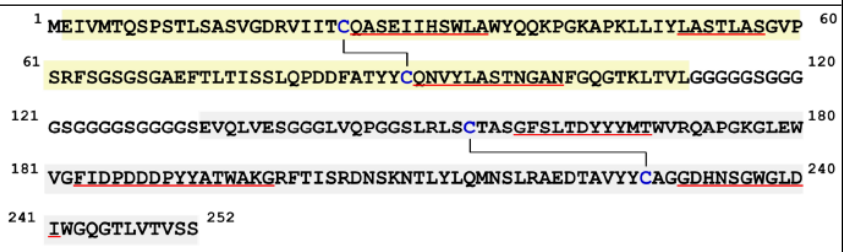
These labeling recommendations considered:

- The 2012 Guidance for Industry ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals[2]
- The 2014 Draft Guidance for Industry: Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products — Content and Format[3]
- The CDER/CBER 2009 Guidance for Industry and Review Staff Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information[4]
- The CDER 2018 Manual of Policy and Procedures (MAPP) 7400.13 Determining the Established Pharmacologic Class for Use in the Highlights of Prescribing Information[5]

[2] ICH S6(R2) accessed via https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s6r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals
[3] The guidance was accessed via: https://www.fda.gov/media/90160/download
[4] The guidance was accessed via: https://www.fda.gov/media/77834/download
[5] MAPP accessed via: https://www.fda.gov/media/86437/download

2 Drug Information

2.1 Drug

CAS Registry Number	1531589-13-5
Generic name:	Brolucizumab
Code names:	ESBA1008 (ESBA 1008) RTH258 (RTH 258) AL-86810 (b) (4)
Chemical name	Humanized monoclonal single-chain Fv (scFv) antibody fragment directed against human vascular endothelial growth factor (hVEGF)
Molecular formula	$C_{1164}H_{1768}N_{310}O_{372}S_8$
Molecular weight	26313 Daltons
Structure or Biochemical Description	
Amino acid sequence (text searchable)	MEIVMTQSPSTLSASVGDRVIITCQASEIIHSWLAWYQQ KPGKAPKLLIYLASTLASGVPSRGSGSGAEFTLTISSLQ PDDFATYYCQNVYLASTNGANFGQGTKLTVLGGGGGS GGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRL SCTAVSGFSLTDYYYMTWVRQAPGKGLEWVGFIDPDD DPYYATWAKGRFTISRDNSKNTLYLQMNSLRAEDTAVY YCAGGDHNSGWGLDIWGQGTLVTVSS
Pharmacologic Class	• vascular endothelial growth factor (VEGF) inhibitor • vascular endothelial growth factor (VEGF) directed antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

- The clinical trials supporting this BLA were conducted under IND 112023; the BLA cross-references the IND.
- The Applicant provided (in BLA module 1.4.2 Statement of Right of Reference) letters of authorization related to manufacturing of the drug product: DMF (b) (4)

2.3 Drug Formulation

The Applicant reports (NDA module 3.2.P.1 Description of the Drug Substance) that the drug product is a sterile, preservative-free, colorless to slightly brownish yellow solution intended for injection. The drug formulation is:

Table 1: Brolucizumab drug product formulation

Ingredient	Concentration	Concentration (mg/ml)	Nominal amount (mg/vial)	Actual amount, (b) (4)	Function
Brolucizumab	12%	120	6		Active pharmaceutical ingredient (API)
Sucrose	5.8%	58	2.9		(b) (4)
Sodium citrate	0.258%	2.58	0.13		
Polysorbate 80	0.02%	0.20	0.01		
(b) (4)					
Water for injection	1 ml				(b) (4)

Table 2: History of drug product formulation

"Initial formulation" ("old formulation")	• Tested in the IND-enabling GLP toxicology study (report # TDOC-0012707) • Tested in Phase 1	(b) (4) sodium citrate, (b) (4)
Formulation A ("new formulation")	• Tested in the 6-month GLP toxicology study (report # TDOC-0016684) and the 3-month GLP toxicology study for the (b) (4) drug substance (report # TDOC-0017689) • Tested in Phase 2 and Phase 3	58 mg/ml sucrose, 2.58 mg/ml sodium citrate, (b) (4) mg/ml polysorbate 80, pH (b) (4)
Formulation B	• The to-be-marketed commercial formulation • Tested in Phase 3 (HAWK extension)	58 mg/ml sucrose, 2.58 mg/ml sodium citrate, 0.2 mg/ml polysorbate 80, pH (b) (4)

- The Applicant provided a summary of the formulation history in the Quality Overall Summary (BLA 3.2.P) [6].
  - The to-be-marketed commercial formulation (formulation B) is slightly modified (b) (4) from the formulation A, which was tested in last two GLP monkey toxicology studies (report # TDOC-0016684 and TDOC-0017689), which was formulation tested Phase 2 and 3 (OSPREY, OWL, STRIKE, HAWK, HARRIER).
  - The Phase 3 product (formulation A) (b) (4)
  - Formulation B was tested in the Phase 3 HAWK extension clinical trial[7], but the exact formulation was not tested in animals.
- At the type B meeting held May 8, 2013 between Alcon and DTOP under IND, the Division had agreed that additional ocular toxicology studies were not warranted to support minor formulation changes.
- For this BLA, this reviewer reaches the same conclusion, that additional nonclinical ocular toxicology studies are not warranted to support the to-be-marketed commercial drug product formulation (Table 1 above).

2.4 Comments on Novel Excipients

- The excipients are qualified for ivt dosing.
- FDA's Inactive Ingredient Search for Approved Drug Products (IID)[8],[9] has no listings for intravitreal (ivt) sucrose. Nonclinical studies submitted to the BLA qualify 5.8% sucrose for ivt dosing.
- (b) (4) The nonclinical studies submitted to the BLA provide additional qualification for 0.258% sodium citrate.
- The IID has one listing for ivt polysorbate 80, 0.015%, for NDA 22048.[11]

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[6] Accessed via: \\cdsesub1\evsprod\bla761125\0001\m2\23-qos\qos-dp-solution-for-injection.pdf

[7] The Clinical Trial Formulae are presented in BLA module 3.2.P.2, accessed via: \\cdsesub1\evsprod\bla761125\0001\m3\32-body-data\32p-drug-prod\rth258-sol-for-inj\32p2-pharm-dev\pharmaceutical-development-clin-trial-formulae.pdf

[8] Public version of the IID accessed via: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm?event=BasicSearch.page

[9] FDA intranet (confidential) version of the IID accessed via: http://intranetapps.test.fda.gov/scripts/iig/ (b) (4)

[11] For NDA 22048, the original 2007 labeling was accessed via: https://www.accessdata.fda.gov/drugsatfda_docs/label/2007/022223,022048lbl.pdf

2.5 Comments on Impurities/Degradants of Concern

P/T identified no safety concerns for impurities or degradants for GMP brolucizumab.

2.6 Proposed Clinical Population and Dosing Regimen

The Applicant has proposed:

- an indication of "treatment of neovascular (wet) age-related macular degeneration (AMD)."
- A dosage of 6 mg (in 50 µl) of brolucizumab, administered by intravitreal injection (b) (4) for the first three doses, (b) (4)

2.7 Regulatory Background

(b) (4)

IND 112023 (the predecessor IND for BLA 761125)	Alcon submitted IND 112023 on 4/20/2011 for ESBA1008 (brolucizumab) for AMD. Sponsorship was formally changed from Alcon to Novartis Pharmaceutical Corporation on 10/16/2018. From a P/T perspective, notable meetings between DTOP and the Sponsor include: • A type B End-of-Phase 2 meeting was held on 5/08/2013 (minutes by Milstein, 6/07/2013) • Type C guidance meetings held on 1/15/2016 (minutes by Milstein, 2/22/2016) and 9/20/2017 (minutes by Milstein, 10/05/2017) • The type B pre-BLA meeting was held 8/27/2018 (minutes by Milstein 9/16/2018)
(b) (4)	

3 Studies Submitted

3.1 Studies Reviewed

Nonclinical study reports were submitted to the BLA under module 4:

Table 3: Primary pharmacology study reports

Study #	Study title
RD-2018-00360	Binding stoichiometry of RTH258 in complex with huVEGF
E1108S031.01	Affinity and potency of ESB1008 revised report
RD-2018-00365	Affinity and potency comparison of brolucizumab with other VEGF antagonists
E1008S030.01	Species cross-reactivity of ESBA1008
E1008S032.01	Isoform selectivity of ESBA1008
TDOC-0013037	Preclinical *in vitro* and *in vivo* efficacy pharmacology of AL-86810, a single-chain anti-VEGF-A antibody fragment

Table 4: Secondary pharmacology study reports

Study #	Study title
ESB01	EpiScreen™ immunogenicity analysis of single chain antibody fragments
ESBA1008	Immunogenicity analysis of ESBA1008-DHP

Table 5: Pharmacokinetic study reports (absorption and distribution)

Study #	Study title	GLP status
TDOC-0012016	Pharmacokinetics of AL-86810 (ESBA1008) following intravenous administration of AL-86810 to non-human primates	no
E1008S029.01	Systemic pharmacokinetics and ocular tissue distribution of anti-VEGF scFv 1008 following a single intravitreal injection to New Zealand White rabbits	no
TDOC-0012998	Ocular pharmacokinetics of AL-86810 following single intravitreal injection of AL-86810 in cynomolgus monkeys – pilot study	no
TDOC-0016874	Ocular pharmacokinetics of AL-86810 (ESBA 1008) in cynomolgus monkeys following intravitreal injection – dose, dose volume and formulation effects	no

Table 6: Single-dose toxicology study reports

Study #	Study title	GLP status
TDOC-0011909	Exploratory intravitreal injection study in naive monkeys with a large-scale batch of ESBA 1008 (AL-86810) with increasing doses of endotoxin	no
TDOC-0015618	An exploratory single dose intravitreal injection ocular toxicity study of various excipients ((b) (4) and sucrose) in New Zealand White rabbits with a four-week observation period	no
TDOC-0017265	AL-86810 (ESBA1008, (b) (4) material): an acute intravitreal screening evaluation in New Zealand White rabbits	no

Table 7: Repeat-dose toxicology study reports

Study #	Study title	GLP status
TDOC-0011462	Exploratory dose range finding intravitreal injection study in naive monkeys with ESBA1008 (AL-86810)	no
TDOC-0012900	Exploratory ocular toxicity evaluation of repeat intermittent intravitreal injections (Q6Wx2) with various batches of AL-86810 (ESBA1008) in cynomolgus monkeys	no
TDOC-0012707	Ocular toxicity evaluation of repeat intermittent intravitreal injections (Q3Wx3) of AL-86810 (ESBA 1008) in cynomolgus monkeys with a 3-week post dose observation period	yes
TDOC-0016684	AL-86810 (ESBA 1008): a six-month intermittent dose (Q4Wx6) intravitreal toxicity study in cynomolgus monkeys with a 3-month interim evaluation	yes
TDOC-0017689	AL-86810 (ESBA 1008, (b) (4) drug substance): A three-month intermittent dose (Q4Wx3) intravitreal toxicity study with a four week observation period in cynomolgus monkeys	yes

3.2 Studies Not Reviewed

Five pharmacokinetic analytical methods and validation reports were submitted to the BLA. Their review is not fully documented herein.

Table 8: PK analytical methods and validation reports

Study #	Study title
TDOC-0013274	Validation of an ELISA method for the determination of AL-86810 (ESBA1008) in cynomolgus monkey serum at (b) (4)

TDOC-0013305	Validation of an ELISA method for the determination of anti-drug antibodies for AL-86810 (ESBA 1008) in cynomolgus monkey serum at (b) (4)
TDOC-0013306	Validation of an ELISA method for the determination of AL-86810 (ESBA 1008) in cynomolgus monkey vitreous humor at (b) (4)
TDOC-0013307	Validation of an ELISA Method for the determination of anti-drug antibodies for AL-86810 (ESBA 1008) in cynomolgus monkey vitreous humor at (b) (4)
TDOC-0013394	Qualification of an ELISA Method for the determination of AL-86810 (ESBA1008) in cynomolgus monkey aqueous humor, neuroretina and RPE-choroid at (b) (4)

- The BLA incorporates the nonclinical study reports submitted to IND 112023, by reference. For IND 112023, the Sponsor submitted (b) (4) This study was not submitted to BLA 761125, and did not test brolucizumab. This reviewer verified that the results of the study do not raise particular concern for brolucizumab's formulation. Review of the study is not further documented herein, and the study report was not relied upon to support the safety of BLA 761125.

3.3 Previous Reviews Referenced

This review references each of the discipline reviews filed under each of the brolucizumab INDs listed above (section 2.7 of this review), including the P/T reviews for:

- (b) (4)
- IND 112023 (Lansita, 5/17/2011; Bebenek, 1/13/2016; McDougal, 8/07/2017)
- (b) (4)
-

4 Pharmacology

4.1 Primary Pharmacology

- Brolucizumab is a humanized single-chain Fv (scFv) antibody fragment with a molecular weight of ~26 kDa which binds to vascular endothelial growth factor A (VEGF-A). Brolucizumab prevents binding of VEGF-A to its receptors, VEGFR1 and VEGFR2, that are expressed on the surface of endothelial cells."

(b) (4)

- IND 112023 was submitted to DTOP on 4/20/2011. The primary pharmacology studies submitted to support the original IND and this BLA were the affinity and potency study (report # E1008S031.01), isoform selectivity (report # E1008S032.01), species cross-reactivity (report # E1008S030.01), and the study showing *in vivo* proof-of-concept (report # TDOC-0013037), reviewed below.
    - A report entitled "**The 3-dimensional structure of ESBA903 in complex with VEGF110**" (report # E903S092.01; study code rdr-sbf-0020-01), dated February 18, 2011 was submitted to the original IND[13] but not to the BLA directly.
        - Because the Applicant explicitly cross-referenced the nonclinical information in IND 112023, P/T considers this report incorporated by reference.
        - The results are consistent with report # RD-2018-00360 (reviewed below).
        - The report was fully reviewed; no safety issues were identified. No further documentation is captured in this review.
    - Four additional study reports were prepared from pharmacology experiments; these were submitted to the BLA (but not the INDs), and were fully reviewed (documentation captured below).

Report title	**Binding stoichiometry of RTH258 in complex with huVEGF**	
Report #	RD-2018-00360	
Key findings	• The Applicant used a simple spectrophotometric assay to verify that one molecule of brolucizumab binds one molecule of VEGF molecule. o *In vivo*, VEGF is a dimer (cysteine-linked); the two VEGFs together bind two VEGFRs, causing signal transduction. o Therefore, the authors remind the reader that two brolucizumab molecules will bind on VEGF dimer. • The calculations needed to verify this conclusion are beyond the scope of this review. P/T did not use this study in reaching a safety conclusion for brolucizumab.	
Report details	Report date	September 13, 2018
	GLP status	No
	Study laboratory	Novartis
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-

[13] Access via the EDR: \\cdsesub1\evsprod\ind112023\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\stf-3-dimensional-structure-of-esba903\3-dimensional-structure.pdf

		rep\421-pharmacol\4211-prim-pd\rd-2018-00360\rd-2018-00360--pre-clinical-study-report.pdf)
Method notes	Test articles	• Brolucizumab, batch # 090916mK002 • Recombinant human VEGF110
	Vehicle	(b) (4) sodium citrate, pH (b) (4)
	Fluorescence detection method	• Brolucizumab has 6 tryptophan residues; huVEGF has none. Tryptophan fluoresces at 290 nm. The author used a spectrophotometer with 290 nm extinction and 340 nm to detect tryptophan. • A single concentration of brolucizumab (50 nM) was used. Aliquots of 1 µM VEGF were added with constant stirring, resulting in a concentration-response curve from 3.3 to 79.2 nM when fluorescence was measured. • Reportedly, results were correct for the concentration and dilution factor. • The authors cite Gabhann and Popel 2007[14] regarding the method. This paper explains that binding of two proteins can affect the intensity of fluorescence to tryptophan and tyrosine, and provides guidance for the calculations needed to quantify the protein interaction.
Results	The author reports a linear relationship (after correction) for the amount of VEGF added up to 59.4 nM, with the curve plateauing at higher concentrations.	

Report title	**Affinity and potency of ESB1008 revised report**
Report #s	• E1108S031.01 • RDR_AssMo_0017-v2
Key findings	• Brolucizumab: o binding to non-glycosylated huVEGF165: K_D = 28.4 pM o binding to glycosylated (more nearly natural) huVEGF165: K_D = 21.6 pM o inhibition of human endothelial cells: IC_{50} = 0.49 nM • *Review note*: the revisions to the report are annotated; this reviewer concludes that the revisions do not affect the results or conclusions.

[14] Groemping Y, Hellmann N (2005) Spectroscopic methods for the determination of protein interactions. Curr Protoc Protein Sci. Chapter 20:Unit 20.8. The paper was not submitted to the BLA, but was available from the National Library of Medicine (NLM), via: https://www.ncbi.nlm.nih.gov/pubmed/18429281

<table>
<tr><td rowspan="4">Report details</td><td>Report date</td><td>September 19, 2019 (for the revised report).</td></tr>
<tr><td>GLP status</td><td>No</td></tr>
<tr><td>Study laboratory</td><td>Novartis</td></tr>
<tr><td>File location</td><td>NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\rd-rrdr-assmo-0017\rd-rrdr-assmo-0017-v2--pre-clinical-study-report.pdf)</td></tr>
<tr><td rowspan="2">Binding assay</td><td>Methods</td><td>• Surface plasmon resonance (SPR) assay, using a Biacore® instrument
• Recombinant human recombinant $VEGF_{165}$ ($huVEGF_{165}$) was obtained commercially (from (b) (4); glycosylated $huVEGF_{165}$ was obtained from a different vendor (b) (4).
o Endogenous $huVEGF_{165}$ is glycosylated; the authors investigated the potential difference.
o Review note: no data regarding the cell source of either $huVEGF_{165}$; not clear if/how well the glycosylation pattern matches humans.
• The mobile phase was brolucizumab or ranibizumab
• Assay temperature not described in the study report (later reporting explain that this study measured binding at 25ºC).</td></tr>
<tr><td>Results</td><td>• Ranibizumab exhibited a higher association rate (k_a) and slower dissociation rate (k_d) compared to brolucizumab. The k_d for ranibizumab was slower than the limit of quantitation; therefore, the K_D could not be calculated.

Table 9: K_D values for brolucizumab and ranibizumab binding to $huVEGF_{165}$ (SPR analysis) (report # E1108S031.01)
<table>
<tr><th>Target</th><th>Test article</th><th>k_a (1/Ms)</th><th>k_d (1/s)</th><th>KD (pM)</th></tr>
<tr><td>$huVEGF_{165}$</td><td>Brolucizumab</td><td>$1.68x10^6$</td><td>$4.78x10^{-5}$</td><td>28.4</td></tr>
<tr><td rowspan="2">Glycosylated $huVEGF_{165}$</td><td>Brolucizumab</td><td>$2.03x10^6$</td><td>$4.40x10^{-5}$</td><td>21.6</td></tr>
<tr><td>Ranibizumab</td><td>$3.35x10^4$</td><td>$< 1x10^{-5}$</td><td>< 298</td></tr>
</table></td></tr>
<tr><td>Binding competition assay</td><td>Methods</td><td>• Recombinant human VEGFR1 or VEGFR2 were bound to the wells of a plate.
• 11 concentrations of brolucizumab or ranibizumab were mixed with an anti-huVEGF antibody and biotinylated huVEGF165, then added to the plates of VEGFR1 or VEGFR2.
• Bound huVEGF165 was detected via ELISA</td></tr>
</table>

<table>
<tr><td></td><td>Results</td><td>• Results are not interpretable – the report did not specify the amount of receptor, huVEGF165, or anti-huVEGF tested.
• The author reports comparable activity for both anti-VEGFs:
o Brolucizumab: EC_{50} = 4.030 nM
o Ranibizumab: EC_{50} = 4.287 nM</td></tr>
<tr><td rowspan="2">Cell proliferation assay</td><td>Methods</td><td>• Human umbilical vein endothelial cells (HUVEC) were stimulated to proliferate with 0.3 nM of non-glycosylated or glycosylated $huVEGF_{165}$
• No details reported for the duration of stimulation, or assay method to measure proliferation</td></tr>
<tr><td>Results</td><td>• Both anti-VEGFs reportedly showed activity (results were reported without supporting data).
• The author reports that non-glycosylated $huVEGF_{165}$ was 2.3-fold more potent for stimulating HUVEC cell growth compared to glycosylated $huVEGF_{165}$.
• The physiological relevance of the difference in activity for brolucizumab competition versus the two $huVEGF_{165}$ forms is unclear.

<table><tr><td></td><td>non-glycosylated $huVEGF_{165}$</td><td>glycosylated $huVEGF_{165}$</td></tr><tr><td>Brolucizumab</td><td>IC_{50} = 0.19 mM</td><td>IC_{50} = 0.49 mM</td></tr><tr><td>Ranibizumab</td><td>IC_{50} = 0.20 mM</td><td>IC_{50} = 0.63 mM</td></tr></table>
Review note: these values appear to be outliers, compared to the other reporting. No supporting data were provided. Because these results are not pivotal to the overall understanding of the mechanism of action, no follow-up is warranted for clarification.</td></tr>
</table>

Report title	**Affinity and potency comparison of brolucizumab with other VEGF antagonists**
Report #	RD-2018-00365
Key findings	These experiments were conducted as a follow-up to report # E1108S031.01 (reviewed above), to compare brolucizumab to approved anti-VEGFs. The results demonstrate proof-of-concept for brolucizumab.
	• For brolucizumab: o Binding to huVEGF at 37°C: K_D = 101 ± 2 pM

		○ Inhibition of human retinal microvascular endothelial cell (HREC) proliferation induced by $VEGF_{165}$: IC_{50} = 54 ± 6 pM ○ Competition for VEGF-A binding to VEGFR2: IC_{50} = 9.8 ± 1.3 pM • Brolucizumab was comparable in potency to aflibercept and ranibizumab in these assays; all three were more potent than bevacizumab.
Report details	Report date	September 27, 2018
	GLP status	No
	Study laboratory	Novartis
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\rd-2018-00365\rd-2018-00365-v2--pre-clinical-study-report.pdf)
Binding assay	Methods	• Surface plasmon resonance (SPR) experiments were performed using a Biacore instrument (from Biacore GE Healthcare). • Recombinant huVEGF165 was immobilized on the chip • The anti-VEGFs were diluted using the (b) (4) [redacted] • The dissociation rate constant (k_d), association rate constant (K_a), and dissociation equilibrium constant (K_D) were calculated. • Experiments were conducted at both 25°C and 37°C [the lower temperature was evaluated to repeat the previous experiment; body temperature was evaluated to obtain more relevant results)
	Results	• The ka values for brolucizumab and aflibercept were two orders of magnitude faster than for bevacizumab and ranibizumab. • The kd values for brolucizumab, aflibercept, and bevacizumab were similar. Ranibizumab's kd value was too slow for the assay to measure (i.e. ranibizumab remains bound to VEGF longer than the other anti-VEGFs). Therefore, K_D values for ranibizumab were only estimates.
	Results:	

Table 10: SPR results for anti-VEGFs at 25°C (report # RD-2018-00365)

Anti-VEGF	k_a (1/Ms)	k_d (1/s)	K_D (pM)	K_D (ng/ml)
Brolucizumab	(2.0±0.2) x 10^6	(4.2±1) x 10^{-5}	21 ± 3	0.546
Ranibizumab	(4.0±0.1) x 10^4	< 1 x 10^{-5}	< 250 ± 7	< 12
Aflibercept	(1.30±0.3) x 10^6	(7.3±9) x 10^{-5}	45 ± 49	4.36
Bevacizumab	(3.7) x 10^4	(6.1) x 10^{-5}	1660	247

Table 11: SPR results for anti-VEGFs at 37°C (report # RD-2018-00365)

Anti-VEGF	K_a (1/Ms)	K_d (1/s)	K_D (pM)	K_D (ng/ml)
Brolucizumab	(2.5±0.1) x 10^6	(2.5±0.1) x 10^{-4}	101 ± 2	2.626
Ranibizumab	(5.8±0.1) x 10^4	< 1 x 10^{-5}	< 171 ± 2	< 8.2
Aflibercept	(3.9±1.8) x 10^6	(1.5±0.1) x 10^{-3}	457 ± 173	44.3
Bevacizumab	(5.9±0.3) x 10^4	(1.9±0.5) x 10^{-4}	3300 ± 1017	491

Review note: the K_D values in units of ng/ml were calculated by this reviewer, using the molecular weight of 26 kDa for brolucizumab (reported by the Applicant), and molecular weights reported in labeling for the others: bevacizumab= 149 kDa; aflibercept = 97 kDa; ranibizumab = 48 kDa. For each protein, X value (in units of pM) multiplied by Y value (weight in kDa) = Z pg/ml. For conversion from pg/ml to ng/ml, multiply by 1000.

Proliferation assay	Methods	• Human retinal microvascular endothelial cells (HREC) were obtained from (b) (4) . o *Review note*: HRECs may be more predictive of anti-VEGF activity on the retina, compared to HUVECs • Media with (b) (4) huVEGF$_{165}$ was used to stimulate cell proliferation over 2 days, in the presence or absence of a series of concentrations of the anti-VEGF test articles.
	Results	All of the anti-VEGFs tested were active for inhibition of VEGF-induced HREC proliferation. The order for potency was aflibercept > ranibizumab > brolucizumab > bevacizumab. **Table 12: Inhibition of VEGF-induced human endothelial cell proliferation (report # RD-2018-00365)** Anti-VEGF | IC_{50} (pM) | IC_{50} (ng/ml)

		Aflibercept: 18 ± 2: 1.7 Ranibizumab: 45 ± 4: 2.1 Brolucizumab: 54 ± 6: 1.4 Bevacizumab: 116 ± 29: 17.2
VEGF-VEGFR2 competition assay	Methods	• VEGFR2 was immobilized in plate wells (concentration not reported). Varying concentrations of the anti-VEGF test articles were mixed with 13 pM of VEGF-A, and then added to the wells for 1, 24, or 48 hours (at room temperature). • After the incubation, VEGF-A binding to VEGFR2 was measured by ELISA.
	Results	• All of the anti-VEGFs were active for inhibition of VEGF-A binding to VEGFR2. Aflibercept and brolucizumab were more potent than ranibizumab and bevacizumab.

Table 13: VEGF competition for VEGFR2 binding (report # RD-2018-00365)

Anti-VEGF	IC_{50} at 1 hour		IC_{50} at 24 hours		IC_{50} at 48 hours	
	pM	ng/ml	pM	ng/ml	pM	ng/ml
Aflibercept	7.7 ± 0.6	0.74	5.5 ± 0.3	0.53	6.1 ± 3.3	0.59
Brolucizumab	9.8 ± 1.3	0.25	6.8 ± 1.0	0.17	6.1 ± 1.0	0.15
Ranibizumab	483 ± 47	23.1	47 ± 3	2.2	19 ± 9	0.91
Bevacizumab	239 ± 30	35.6	32 ± 1	4.7	39 ± 19	5.8

Report title	**Isoform selectivity of ESBA1008**	
Report #	E1008S032.01	
Key findings	The three major isoforms of huVEGF are $huVEF_{165}$, $huVEGF_{121}$, and $huVEGF_{110}$. Brolucizumab bound all three isoforms of huVEGF with comparable potency. • EC_{50} for $huVEF_{165}$ =28.4 pM • EC_{50} for $huVEF_{110}$ =25.2 pM • EC_{50} for $huVEF_{121}$ =34.1 pM	
Report details	Report date	October 20, 2010
	GLP status	No
	Study laboratory	ESBATech
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-

<table>
<tr><td></td><td></td><td>rep\421-pharmacol\4211-prim-pd\rd-rrdr-assmo-0016\rd-rrdr-assmo-0016—pre-clinical-study-report.pdf)</td></tr>
<tr><td>Methods notes:</td><td colspan="2">• SPR analysis, with the VEGF immobilized; anti-VEGF in the analyte.
• Buffer was (b) (4) Assay temperature not reported.</td></tr>
<tr><td>Results:</td><td colspan="2">• As was observed previously, ranibizumab exhibited a faster k_a and slower k_d than brolucizumab. Ranibizumab's kd was slower than the limit of quantitation; therefore the K_D values for ranibizumab are only estimates.

Table 14: VEGF isoform selectivity of brolucizumab (report # E1008S032.01)
<table>
<tr><th>Anti-VEGF</th><th>VEGF</th><th>K_a (1/Ms)</th><th>K_d (1/s)</th><th>K_D (pM)</th><th>K_D (ng/ml)</th></tr>
<tr><td rowspan="3">Brolucizumab</td><td>$huVEF_{165}$</td><td>1.68 $x10^6$</td><td>4.78 $x10^{-5}$</td><td>28.4</td><td>0.738</td></tr>
<tr><td>$huVEF_{110}$</td><td>1.45 $x10^6$</td><td>3.66 $x10^{-5}$</td><td>25.2</td><td>0.655</td></tr>
<tr><td>$huVEF_{121}$</td><td>1.66 $x10^6$</td><td>5.67 $x10^{-5}$</td><td>34.1</td><td>0.886</td></tr>
<tr><td rowspan="3">Ranibizumab</td><td>$huVEF_{165}$</td><td>3.34 $x10^4$</td><td><1 $x10^{-6}$</td><td>< 29.9</td><td>< 1.43</td></tr>
<tr><td>$huVEF_{110}$</td><td>4.10 $x10^4$</td><td><1 $x10^{-6}$</td><td>24.4</td><td>< 1.17</td></tr>
<tr><td>$huVEF_{121}$</td><td>4.94 $x10^4$</td><td><1 $x10^{-6}$</td><td>20.2</td><td>< 0.969</td></tr>
</table>
• The author notes that the results for ranibizumab are consistent with those reported in the literature by Lowe et al. 2007[15] for all three VEGF isoforms; this reviewer concurs.</td></tr>
</table>

Report title	**Species cross-reactivity of ESBA1008**
Report #	E1008S030.01
Key findings	• Brolucizumab binds human, mouse, rat, dog, pig, and cat VEGF with comparable affinity. Brolucizumab did not bind rabbit VEGF. • The authors report that cynomolgus monkey VEGF has the same amino acid sequence as human VEGF; therefore, monkey VEGF was not tested in these experiments.
	• *Review note*: these results are not adequate to rule out any species as not pharmacologically relevant (i.e. rabbit), or to demonstrate pharmacological relevance (e.g. rat).

[15] Lowe J, Arujo J, Yang J, Reich M, Oldendorp A, Shiu V, Quarmby V, Lowman H, Lien S, Gaudreault J, Maia M. 2007. Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor *in vitro* and *in vivo*. Exp. Eye. Res. 85(4):425-430. Paper accessed via https://www.ncbi.nlm.nih.gov/pubmed/17714704

	• DTOP considers adequate characterization important for binding of anti-VEGFs to each isoform of VEGF-A commonly expressed in patients. These experiments only evaluated one VEGF-A isoform per species. The literature reports that variants have been detected for animal VEGFs. Therefore, the potential of brolucizumab to bind other isoforms in the nonclinical models is unknown. Further characterization of binding (or lack of binding) to the other common isoforms would be important, prior to considering non-primate models for further nonclinical testing (if needed to support future indications).	
Report details	Report date	October 20, 2010
	GLP status	No
	Study laboratory	ESBATech
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\rd-rrdr-assmo-0007\rd-rrdr-assmo-0007—pre-clinical-study-report.pdf)
ELISA binding assay	Methods	• Wells were coated with 0.5 µg/ml of VEGF from the test species: human, cat, mouse, pig, and rabbit [rat and monkey VEGF not tested] • After blocking, four concentrations of brolucizumab were tested: 0, 5, 50, 500, and 5000 ng/ml. • As a positive control, a recombinant chimeric protein consisting of human VEGFR2 + a Fc region was used (huVEGFR2/Fc).
	Results	• Brolucizumab binding to human, mouse, cat and pig VEGF appeared comparable: 5 ng/ml gave a less-than-maximal response, and higher doses gave a maximal response. • Brolucizumab bound rabbit VEGF weakly at 500 and 5000 ng/ml (dose response apparent). The authors concluded that the rabbit is not a pharmacologically relevant model, and this reviewer concurs.
SPR binding assay	Methods	• SPR using a Biacore® instrument was conducted. • For each test species, VEGF was immobilized on the chip. Increasing concentrations of brolucizumab were added to the analyte.
	Results	• Brolucizumab bound VEGF from multiple species with comparable potency, excepting that no binding to rabbit VEGF was detected. **Table 15: Species screen for brolucizumab binding to VEGF (by SPR) (report # E1008S030.01)**

		<table><tr><th>VEGF form</th><th>K_D (pM)</th><th>K_D (ng/ml)</th></tr><tr><td>Human $VEGF_{165}$</td><td>28.4</td><td>0.738</td></tr><tr><td>Mouse $VEGF_{164}$</td><td>45.3</td><td>1.177</td></tr><tr><td>Rat $VEGF_{164}$</td><td>36.5</td><td>0.949</td></tr><tr><td>Dog $VEGF_{163}$</td><td>34.6</td><td>0.899</td></tr><tr><td>Human $VEGF_{110}$</td><td>25.2</td><td>0.655</td></tr><tr><td>Cat $VEGF_{110}$</td><td>30.8</td><td>0.800</td></tr><tr><td>Pig $VEGF_{110}$</td><td>58.1</td><td>1.51</td></tr><tr><td>Rabbit $VEGF_{110}$</td><td>No binding</td><td>No binding</td></tr></table>
	Notes	• Reportedly, VEGF-A has twelve known isoforms in the rat[16], nine known isoforms in the mouse[17], three known isoforms in the dog[18], and six known isoforms in the cynomolgus monkey[19]. • For the rabbit, NCBI[20] only reports one isoform, VEGF165. Hofstaetter et al. 2007[21] report on 4 rabbit isoforms (VEGF121, VEGF165, VEGF183, and VEGF189). Therefore, it is not clear that testing only rabbit VEGF110 demonstrates that the rabbit would not be a pharmacologically relevant model.

[16] Gene. Vegfa vascular endothelial growth factor A [Rattus norvegicus (Norway rat)]. Gene ID: 83785. Version updated July 2, 2019. National Center for Biotechnology Information (NCBI). Accessed via: https://www.ncbi.nlm.nih.gov/gene/83785
[17] Gene. Vegfa vascular endothelial growth factor A [Mus musculus (house mouse)]. Gene ID: 22339. Version updated July 23, 2019. NCBI. Accessed via: https://www.ncbi.nlm.nih.gov/gene/22339
[18] Gene. VEGFA vascular endothelial growth factor A [Canis lupus familiaris (dog)]. Gene ID: 403802. Version updated May 12, 2019. NCBI. Accessed via: https://www.ncbi.nlm.nih.gov/gene/403802
[19] Gene. VEGFA vascular endothelial growth factor A [Macaca fascicularis (crab-eating macaque)]. Gene ID: 102140241. Version updated September 21, 2018. NCBI. Accessed via: https://www.ncbi.nlm.nih.gov/gene/102140241
[20] Gene. VEGFA vascular endothelial growth factor A [Oryctolagus cuniculus (rabbit)] Gene ID: 100008899. Version updated June 14, 2019. NCBI. Accessed via: https://www.ncbi.nlm.nih.gov/gene/100008899
[21] Hofstaetter JG, Saad FA, Sunk IG, Bobacz K, Friehs I, Glimcher MJ. 2007. Age-dependent expression of VEGF isoforms and receptors in the rabbit anterior cruciate ligament. Biochim Biophys Acta.1770(7):997-1002. Accessed via: https://www.ncbi.nlm.nih.gov/pubmed/17459591

<table>
<tr><td>Report title</td><td colspan="2">Preclinical in vitro and in vivo efficacy pharmacology of AL-86810, a single-chain anti-VEGF-A antibody fragment</td></tr>
<tr><td>Report #</td><td colspan="2">TDOC-0013037</td></tr>
<tr><td>Key findings</td><td colspan="2">• Proof-of-concept for the anti-VEGF activity of brolucizumab was demonstrated in vitro [human retinal endothelial cells (HRECs), bovine retinal endothelial cells (BRECs)] and in vivo (rats and mice).
• For the rodent studies, no ocular or systemic safety endpoints were included.</td></tr>
<tr><td rowspan="4">Report details</td><td>Report date</td><td>January 25, 2011</td></tr>
<tr><td>GLP status</td><td>No</td></tr>
<tr><td>Study laboratory</td><td>Alcon Research Ltd.
6201 S. Freeway, Fort Worth, Texas 76134</td></tr>
<tr><td>File location</td><td>NDA module 4.2.1.1 Primary Pharmacodynamics</td></tr>
<tr><td rowspan="3">In vitro assays with human and retinal endothelial cells</td><td>HREC migration assay methods</td><td>• Human retinal endothelial cells (HRECs) were seeded into fibronectin-coated chemotaxis plate wells with 10 ng/ml of recombinant huVEGF$_{165}$ to stimulate migration across a filter (8 µm pore size)
o Media included [redacted] (b) (4); the amount of VEGF in the [redacted] (b) (4) was not assessed.
• Brolucizumab or ranibizumab were tested over a range of concentrations (0.01 to 3 nM).</td></tr>
<tr><td>BREC proliferation assay methods</td><td>• Bovine retinal endothelial cells (BRECs) were cultured with 50 ng/ml of recombinant huVEGF$_{165}$ to stimulate proliferation.
o Media included [redacted] (b) (4)). The amount of VEGF in the [redacted] (b) (4) was not assessed.
• Brolucizumab or ranibizumab were tested over a range of concentrations (0.06 to 3.3 nM).</td></tr>
<tr><td>Results</td><td>• Both anti-VEGFs were active; brolucizumab appeared to be more potent.

Table 16: Comparison of brolucizumab and ranibizumab for in vitro inhibition of HREC migration and BREC proliferation (report # TDOC-0013037)
<table>
<tr><td rowspan="2">Assay</td><td colspan="2">Brolucizumab</td><td colspan="2">Ranibizumab</td></tr>
<tr><td>nM</td><td>ng/ml</td><td>nM</td><td>ng/ml</td></tr>
<tr><td>HREC migration IC$_{50}$</td><td>0.093 ± 0.02</td><td>2.41</td><td>0.11 ± 0.09</td><td>2.86</td></tr>
<tr><td>BREC proliferation IC$_{50}$</td><td>0.77 ± 0.47</td><td>20.0</td><td>0.64 ± 0.28</td><td>30.7</td></tr>
</table></td></tr>
</table>

		• The study authors reported results on a nM basis. This reviewer calculated the results on a ng/ml basis, using the molecular weights of 26 kDa for brolucizumab, and 48 kDa for ranibizumab. • *Review note*: these IC_{50} values are remarkably higher than values calculated from other experiments (i.e. apparently less potent for these two experiments). Because the *in vitro* conditions may not reflect *in vivo* physiology, these results are more useful to compare the two products side-by-side, and might underpredict *in vivo* activity.
In vivo (adult rat) assay:	Overall finding	• The goal of the assay was to measure inhibition of retinal vascular permeability by brolucizumab. • Based on reporting deficiencies for the methods and results, this reviewer concludes that the experiments are not interpretable. o The authors reported that intraocular hemorrhage and mortality occurred. Attribution to the procedure versus treatment is unclear. o Vascular permeability was not directly assessed. • This experiment was not pivotal for understanding the pharmacology and toxicology of brolucizumab; the results are not relied upon to support the safety of this BLA.
	Summary	• The authors injected the eyes of adult male Sprague-Dawley rats with 10 µg of bovine serum albumin (BSA) + 0.5 µg of $rhVEGF_{165}$, to induce retinal vascular leakage; 24 hours later, rats were dosed intravenously with Evans blue dye (intended to quantify plasma leaking from retinal vessels) and sacrificed. • Eyes were pre-dosed with brolucizumab or ranibizumab 1, 3, 7, or 14 days prior to the BSA+VEGF, with the goal of inhibiting vascular leakage. • The authors concluded that brolucizumab and ranibizumab were active when given 1 day prior to BSA+VEGF challenge, but were not active when given 14 days prior.
In vivo (rat pup) assay	Methods	• Pregnant Sprague-Dawley rats were allowed to litter; room air was 50% oxygen for postpartum day (PPD) 0-14; normal air from PPD14-20.

		• Pup eyes were dosed with 0, 5 to 300 µg/eye brolucizumab on various days, beginning PPD 14 (ivt volume 5 µl) • Retinas were harvested on PPD 20 or 21. A retinal neovascularization score was made for each eye (no details on how this scoring was done). • The authors considered the early dosing timepoints to be an oxygen-induced retinopathy (OIR) model, and later dosing to be a "preretinal neovascularization (NV)" model
	Results	• Brolucizumab showed activity when dosed at early time points (up to PPD 17), but not thereafter (i.e. beginning dosing at PPD 17). • It is not clear from the study report how severely the eyes were affected by the model (results were only reported normalized to controls). Therefore, it is not clear that the model was adequate to measure brolucizumab pharmacology. • The relevance of these experiments to patients with AMD is unclear.
In vivo (adult mouse) assay: laser-induced choroidal neovascular-ization (CNV)	Methods	• The retina of adult C57ZBL/6J mice received 3 to 4 laser burns/eye, using a slit lamp, as a model for CNV. o Observation of a bubble under the retina was considered evidence that Bruch's membrane had been successfully ruptured by the laser. o The intensity/duration of the laser treatment was not reported. • Different groups of mice were dosed with brolucizumab by daily intraperitoneal injection (100 µl/mouse volume) or by intravitreal injection (2 µl/eye volume) • At sacrifice, mice were perfused with fluorescein-labeled dextran to better visualize the retinal vasculature. Automated image analysis software was used to calculate the mean and median area of hyper fluorescence, as an estimate of CNV.
	Results	Brolucizumab exhibited activity for decreasing CNV lesion size compared to vehicle. • Daily intraperitoneal brolucizumab exhibited a dose-response. • Ivt brolucizumab was active, but did not exhibit a clear dose response. A positive control (AL-39324, a small molecule tyrosine kinase inhibitor of

VEGFR, tested at a single dose level) showed more activity, suggesting that brolucizumab target saturation had not been achieved.

Table 17: Brolucizumab (intravitreal and intraperitoneal injections) reduced choroidal neovascularization following laser in mouse eyes (report # TDOC-0013037)

Day of retinal evaluation after laser burn	Route	Dose	CNV area mean change from vehicle control
7	Ivt on D0	45 µg/eye	-17.2%
		130 µg/eye	-35.2%
14	Ivt on D0 and D7	15 µg/eye	-21.3%
		45 µg/eye	-27.6%
		130 µg/eye	-31.3%
14	IP daily from D-1 to D14	32 mg/kg	-35.0%
		320 mg/kg	-47.4%

4.2 Secondary Pharmacology

- The Applicant submitted two reports for *in vitro* experiments conducted to predict potential immunogenicity of brolucizumab under primary pharmacology. However, P/T considers these secondary pharmacology assays (because immunogenicity is not part of the intended pharmacology).
- These results were important prior to clinical testing, in order to characterize safety.

Report title	**EpiScreen™ immunogenicity analysis of single chain antibody fragments**	
Report #	• ESB01 • E903S022.01	
Key findings	Two sequences (one 8 amino acids, the other 10 amino acids) from the amino acid sequence of brolucizumab were identified as potentially immunogenic, using a screening assay with human peripheral blood mononuclear cells (PBMCs) *in vitro*	
Report details	Report date	August 26, 2008
	GLP status	No

	Study laboratories	• (b) (4) • (b) (4) • ESBATech AG, Wagistrasse 21, CH-8952 Schlieren, Switzerland
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\e903s022\rd-e903s022—pre-clinical-study-report.pdf)
Method details	Episcreen™ assay rationale	• In the normal adaptive immune system, when resting CD4+ T cells recognize specific antigens presented by mature antigen presenting cells, the T cell becomes activated, and activate B cells to become antibody producing plasma cells. • Using a panel of PBMCs depleted of CD8+ T cells, the induction of proliferation of CD4+ T cells is considered evidence of potential T-cell mediated immunogenicity
	Episcreen™ assay design	• A panel of PBMCs from 50 different donors, selected to maximize coverage of known human HLA-DR alleles, was used • Assay controls included keyhole limpet hemocyanin (KLH), and two viral proteins as known antigens • Cells were incubated with the polypeptide sequences for 6 days. Proliferation was assessed by radio-thymidine incorporation.
	Test articles	• Brolucizumab is a single chain of 252 amino acids. • Using the amino acid sequence of brolucizumab, 125 polypeptides of 14 or 15 amino acids were synthesized (with overlapping regions). Mutations were introduced at specific positions, to isolate the region of immunogenicity (i.e. a length of 14 of 15 amino acids is needed for antigenicity
Results	• Three sequences were considered positive o Each were positive in 3/50 PMBC samples (6%) from different donors (i.e. no donor gave a positive response for more than one of the sequences). o The authors consider positive responses in 3/50 donors to be weak signals. o The positive sequences were (with mutations from brolucizumab bolded): ▪ DFATYYCQ**NVYLAST** ▪ **TWA**KGRFTISRD**T**SK ▪ KGRFTISRD**T**SKNT**V** • This reviewer notes “DFATYYCQNVYLAST” begins at amino acid 82 of the brolucizumab sequence and “KGRFTISRD” begins at amino acid 197 of the brolucizumab sequence.	

	• The authors note that the sequence "KGRFTISRD" was in their database as having previously been flagged as positive in a separate experiment (i.e. screening a different antibody).

Report title	**Immunogenicity analysis of ESBA100-DHP**	
Report #s	• ESBA1008 • E1008S034.02	
Key findings	• Using the same T-cell proliferation assay described above (report # ESB01), 13/83 different peptides (covering a substantial portion of the amino acid sequence of brolucizumab) were positive in PBMCs from one donor, but none were positive in PBMCs from multiple donors. • The authors consider this response to be weak and non-concerning. This reviewer does not concur. The positive findings in this *in vitro* assay were consistent with the relationship between treatment-emergent adverse events (TEAEs) and intraocular inflammation (IOI) observed in patients treated with brolucizumab.	
Report details	Report date	January 3, 2011
	GLP status	no
	Study laboratory	ESBATech (a Novartis Company)
	File location	NDA module 4.2.1.1 Primary Pharmacodynamics (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\421-pharmacol\4211-prim-pd\rd-rrdr-invhi-0023\rd-rrdr-invhi-0023—pre-clinical-study-report.pdf)
Method notes	• A total of 83 15-mer peptides were made, with overlapping sequences, to cover the entire amino acid sequence of brolucizumab. No mutations were introduced. • PBMCs from 50 healthy donors were used, with CD8+ T cells depleted. The proliferation assay measured BrdU incorporation.	
Results:	• As noted above, 13/83 sequences were positive. o The sequences are presented in the study report (not copied into this review because no particular sequence was highlighted as more important/positive than others). • Notably, neither of the sequences previously identified (in report # ESB01) were positive in any PBMC sample in this assay. • The Applicant (NDA module 2.6.2 Pharmacology Written Summary) considers the concern from these experiments "low" and superseded by clinical data. This reviewer agrees that clinical data supersede these data, but does not agree that the concern raised is low (i.e. these experiments appear have correctly predicted ocular inflammation in patients).	

	o The Applicant reports (NDA module 2.5 Clinical Overview) treatment-emergent ADA was associated with increased incidence of intraocular inflammation, but not "non-ocular AEs including systemic hypersensitivity". o P/T defers to the Clinical and Clinical Pharmacology disciplines regarding interpretation of the clinical results.

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Report title	**Pharmacokinetics of AL-86810 (ESBA1008) following intravenous administration of AL-86810 to non-human primates**	
Report #s	• TDOC-0012016 • P-10-508	
Key findings	• 6/sex cynomolgus monkeys received a single intravenous doses of 2 mg/kg brolucizumab. Serum TK was evaluated using a quantitative ELISA method with a lower limit of quantitation (LLOQ) of 2 ng/ml. Anti-drug antibodies (ADA) were measured using a qualitative ELISA method. • Clearance after iv injection was rapid (as expected for a single-chain 252 amino acid protein): initial $t_{1/2}$ < 30 minutes; terminal $t_{1/2}$ of 1.5 hours. • No safety endpoints were assessed	
Report details	Report date	March 31, 2011
	GLP status	No
	Study laboratory	• In-life: Alcon Research, Ltd., 6201 S. Freeway, Fort Worth, Texas 76134 • Analytical work: (b) (4)
	File location	BLA module 4.2.2.2 Absorption (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4222-absorp\dmpk-rtdoc-0012016\dmpk-rtdoc-0012016—pre-clinical-study-report.pdf)
Method details	Test article	Brolucizumab (AL-86810), ID # 117086 (purity not reported)
	Formulation:	• (b) (4) • Note: this is the "old formulation" used for Phase 1

	Blood sampling	• 14 timepoints after dosing for TK, including D7 (168 hours) and D15 (360 hours) • 3 time points for ADA: pre-dose, D7, and D15

PK results:

- One male was an outlier (# 1004), and was removed from analysis. The C_0 value for this animal was only 652 ng/ml, and the T_{max} was at 4 hours (1800 ng/ml), suggesting that misdosing occurred. This animal was negative for ADA at all timepoints (pre-dose, D7, D15). This reviewer concurs with removal of this animal's TK data from analysis.
- For the other monkeys, exposure was slightly higher for females.

Table 18: Serum TK parameters following a single intravenous dose (2 mg/kg) to cynomolgus monkeys (report # TDOC-0012016)

Parameter	Males	Females	Both sexes combined
C_0 (ng/ml)	52,200 ± 9860	64,800 ± 14,900	57,700 ± 14,100
Initial $t_{1/2}$ (h)	0.514 ± 0.073	0.464 ± 0.017	0.483 ± 0.047
Terminal $t_{1/2}$ (h)	6.70 ± 0.61	5.11 ± 1.39	5.64 ± 1.50
C_L (L/h)	0.427 ± 0.087	0.302 ± 0.179	0.352 ± 0.157
V_z (L/kg)	0.575 ± 0.163	0.515 ± 0.193	0.539 ± 0.175
$AUC_{0\text{-inf}}$ (ng*h/ml)	35,700 ± 6870	39,300 ± 27,900	37,900 ± 21,300

Results presented as mean ± standard deviation

ADA results:	• One monkey (male # 1003) was positive for ADA prior to dosing. • No monkeys developed new ADA after dosing.

Report title	**Systemic pharmacokinetics and ocular tissue distribution of anti-VEGF scFv 1008 following a single intravitreal injection to New Zealand White rabbits**	
Report #	E1008S029.01	
Key findings	• Single-dose ivt TK study in rabbits • Distribution: vitreous humor > retina > RPE-choroid > aqueous humor > serum • Elimination $t_{1/2}$ from ocular tissues was 2.4 to 3.4 days. • Severe ocular inflammation was observed in 4/36 rabbits treated at a single dose-level, 500 µg/eye o Based on the timing of the study, presumably the pre-GMP pilot batch material was used	
Report details	Report date	December 22, 2010
	GLP status	No

	Study laboratory	(b) (4)
	File location	BLA module 4.2.2.3 Distribution (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4223-distrib\dmpk-re1008s029\dmpk-re1008s029-pk—pre-clinical-study-report.pdf)
Methods	Test article	Brolucizumab (anti-VEGF scFV 1008), batch # 090916MK004 • Purity 100% • Endotoxin ≤ 0.02 EU/mg
	Formulation	• (b) (4) mM sodium citrate, (b) (4) • *Review note*: (b) (4) in the drug product formulation.
	Dosing	36 male New Zealand White (NZW) rabbits received a single unilateral (OD) intravitreal (ivt) dose of 500 µg/eye brolucizumab. The dosing volume was 50 µl.
	Sampling	• Serial sacrifice to 288 hours post-dose (D12). TK parameters for the aqueous humor, retina, RPE-choroid, serum, and vitreous humor were calculated. • ADA was not evaluated for any of the animals
	Safety endpoints	Slit-lamp and indirect ophthalmoscopy were conducted for all rabbits pre-dose, and for rabbits being necropsied at 24, 72, 144, and 288 hours post-dose.
Ophthalmoscopy results	• "In general", treatment caused "mild, transient anterior segment inflammatory response that largely resolved by 72 hours postdose" • Four treated eyes exhibited severe inflammation (initially noticed at 24 hours post-dose). These animals were replaced (to obtain TK from eyes without severe inflammation)	

TK results:

Table 19: Ocular TK for the single-dose ivt rabbit study (report # E1008S029.01)

Parameter	Units	Aqueous humor	Retina	RPE-choroid	Serum	Vitreous humor
T_{max}	h	24	1	1	24	1
C_{max}	ng/ml	35870	137025	79422	12.53	415061
	µg/ml	35.8	137.0	79.4	0.012	415.0
$t_{1/2}$	h	65.2	82.4	76.7	59.3	70.6

AUC_{0-t}	ng*h/ml	4250442	15765614	8423104	1263	41922345
	mg*h/ml	4.25	15.76	8.42	0.00126	41.92
AUC_{0-inf}	ng*h/ml	4487944	17327386	9086298	1316	44805439
	mg*h/ml	4.48	17.32	9.08	0.00131	44.80

- Authors reported C_{max} and AUC in units of ng/ml and ng*hr/ml. This reviewer calculated the adjusted values.

Report title	**Ocular pharmacokinetics of AL-86810 following single intravitreal injection of AL-86810 in cynomolgus monkeys – pilot study**	
Report #s	• TDOC-0012998 • PKDM 1583	
Key findings	• 14 cynomolgus monkeys received a single unilateral (OD) ivt dose of 1000 µg of brolucizumab (AL-86810), volume of 50 µl. • Distribution: central retina > peripheral retina > vitreous humor > aqueous humor > central choroid > peripheral choroid > serum • Authors report no distribution from the dosed eye to the undosed eye (OS), LLOQ= 2 ng/ml • Elimination $t_{1/2}$ from ocular tissues was 2.0 to 3.2 days. • No specific safety endpoints measured; the authors report that the animals remained in good health	
Report details	Report date	April 06, 2011
	GLP status	No
	Study laboratory	• In-life: Alcon Research Ltd., 6201 S. Freeway, Fort Worth, Texas 76134 • Sample analysis: (b) (4)
	File location	BLA module 4.2.2.3 Distribution (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4223-distrib\dmpk-rtdoc-0012998\dmpk-rtdoc-0012998—pre-clinical-study-report.pdf)
Methods	Test article	• Brolucizumab (AL-86810), lot # ESBA1008-10802-20
	formulation	• (b) (4) • Purity ≥ 95.18% • Endotoxin = 0.063 EU/mg
	Species	• A total of 7 male and 7 female cynomolgus monkeys were used.
	Dosing and sampling	• Monkeys received a single ivt OD dose of 1000 µg of brolucizumab. • Sacrifice of 1/sex/timepoint: 1, 6, 12, 24, 72, 168, and 336 hours post-dose.

		• Tissues/matrix collected at sacrifice: aqueous humor, vitreous humor, central retina, peripheral retina, central choroid, and peripheral choroid were measured for both eyes.

TK results:

- Authors report rapid distribution of the administered dose from the vitreous into the retina, with < 1% remaining in the vitreous by D14

Table 20: Ocular distribution for the single-dose ivt monkey study (report # TDOC-0012998)

Parameter	Unit	Aqueous humor	Central Choroid	Peripheral choroid	Central retina	Peripheral retina	Vitreous humor	Serum
T_{max}	h	6	24	6	12	6	1	6
$t_{1/2}$	h	56.0	54.7	59.2	50.9	58.1	50.1	77.9
C_{max}	ng/ml	287,000	153,000	195,000	598,000	160,000	439,000	60.9
	µg/ml	287	153	195	598	160	439	0.060
	µM	10.9	5.81	7.41	22.7	17.5	16.7	0.0023
$AUC_{0-336\ h}$	ng*h/ml	12	1.2e7	1.28e7	3.97e7	3.41e7	3.16e7	3430
	mg*h/ml	12	12.8	13	39.7	34.1	31.6	0.0034
	µM*h	456	486	494	1510	1300	1200	0.130

- Aqueous humor, vitreous humor, and serum units are ng/ml; choroid and retina are actually ng/g. The different denominators are not captured in the table above (to avoid confusion)
- Authors reported C_{max} in units of ng/ml and µM, and AUC in units of ng*h/ml and µM*h. This reviewer calculated the units on a µg/ml and µg*h/ml basis.

ADA:

- Blood for ADA was collected from the last 2 monkeys only (pre-dose, and at the 336 hour time point).
- One of the two monkeys had pre-dose ADA.

Report title	**Ocular pharmacokinetics of AL-86810 (ESBA 1008) in cynomolgus monkeys following intravitreal injection – dose, dose volume and formulation effects**
Report #s	• TDOC-0016874 • P-13-503
Key findings	• Cynomolgus monkeys received a single bilateral ivt dose of 1 or 6 mg/eye brolucizumab. The right eye (OD) was dosed prior to the left eye (OS) at staggered time points to maximize the number of time points assessed (this approach presumes no cross-over). • Distribution was vitreous humor > peripheral retina > central retina > peripheral choroid > central choroid > serum

<table>
<tr><td></td><td colspan="2">• No safety data reported</td></tr>
<tr><td rowspan="4">Report details</td><td>Report date</td><td>September 16, 2014</td></tr>
<tr><td>GLP status</td><td>No</td></tr>
<tr><td>Study laboratory</td><td>Alcon Research Ltd.
6201 S. Freeway, Fort Worth, Texas 76134</td></tr>
<tr><td>File location</td><td>BLA module 4.2.2.3 Distribution (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4223-distrib\dmpk-rtdoc-0016874\dmpk-rtdoc-0016874—pre-clinical-study-report.pdf)</td></tr>
<tr><td rowspan="4">Method details</td><td>Test article</td><td>Brolucizumab (RTH258, AL-86810, eSBA1008)</td></tr>
<tr><td>Formulation</td><td>• Formulation A (new formulation): [redacted] (b) (4) sucrose, [redacted] (b) (4) polysorbate 80, pH [redacted] (b) (4)
• Initial formulation (old formulation): [redacted] (b) (4)</td></tr>
<tr><td>Species</td><td>21/sex cynomolgus monkeys</td></tr>
<tr><td>Dosing:</td><td>• 5 different groups were tested, to vary the ivt volume and dose
<table>
<tr><th>Group #</th><th>Dose (mg/eye)</th><th>Dose volume (µl/eye)</th></tr>
<tr><td>1</td><td>6</td><td>50</td></tr>
<tr><td>2</td><td>6</td><td>100</td></tr>
<tr><td>3</td><td>6</td><td>100</td></tr>
<tr><td>4</td><td>1</td><td>50</td></tr>
<tr><td>5</td><td>1</td><td>8.5</td></tr>
</table>
• Staggering of OD and OS doses to achieve a total of 6 timepoints:
o 3/sex/group were dosed OD 504 hours and OS 1 hour prior to sacrifice.
o 3/sex/group were dosed OD 336 hours and OS 6 hours prior to sacrifice.
o 3/sex/group were dosed OD 168 hours and OS 24 hours prior to sacrifice.</td></tr>
<tr><td>Results</td><td colspan="2">• The authors concluded that changing the dose volume and formulation did not demonstrate meaningful changes in ocular distribution. This reviewer concurs.
• Brolucizumab distributed from the vitreous into the retina > choroid > aqueous humor > serum [C_{max} and AUC values provided in the study report, but not copied into this review].

Table 21: Selected ocular distribution parameters for the single-dose OU ivt monkey study (report # TDOC-0016874)</td></tr>
</table>

Matrix	T_{max} (h)	T_{last} (h)	$t_{1/2}$ (h)
Vitreous humor	1	504	40.5 to 62.4
Peripheral retina	6 to 24	504	47.4 to 61.9
Central retina	1 to 24	24 to 504	50.5 to 62.0
Peripheral choroid	1 to 6	504	21.8 to 59.9
Central choroid	6 to 24	168 to 504	52.7 to 73.6
Aqueous humor	6	504	42.9 to 52.8
Serum	1 to 6	24 to 336	42 to 54.9

Group 1's test article ((b) (4) % brolucizumab; 6 mg in 50 µl) was the closest to the clinical drug product. BLA module 2.6.4 (Pharmacokinetic Written Summary) documents that this formulation was the same as used in clinical trial # C-12-006.

Table 22: Ocular distribution data (AUC and Cmax) for Group 1 (12% brolucizumab) for the single-dose OU ivt monkey study (report # TDOC-0016874)

Matrix	C_{max} (µg/g or µg/ml)	AUC0-$_{last}$ (µg*h/g or µg*h/ml)
Vitreous humor	1960	173,000
Peripheral retina	1750	121,000
Central retina	635	74,300
Peripheral choroid	1120	99,000
Central choroid	284	20,100
Aqueous humor	1240	77,100
Serum	0.564	31.3

6 General Toxicology

- All of the toxicology studies tested the intravitreal (ivt) route of exposure; no testing by other routes was performed.
- Under IND, the first ivt toxicology study conducted was a non-GLP dose-range finding study (DRF) in cynomolgus monkeys (report #s TDOC-0011462; E-10-003). The drug substance was contaminated with endotoxin, resulting in ocular inflammation. The initial follow-up study was a non-GLP single-dose study in monkeys (report #s TDOC-0011909, E-10-29), followed by another non-GLP repeat-dose study in monkeys (report #s TDOC-0012900, E-10-043).
- The pivotal IND-enabling GLP-repeat-dose monkey ivt toxicology study (report # TDOC_0012707) used drug substance manufactured in compliance with good

manufacturing practices (GMP); the high-dose (6 mg/eye) supported initiation of the first-in-human clinical trial (C-10-083).

- The GLP chronic toxicology study (Q4Wx6) was conducted in monkeys (report # TDOC-0016684), and included a formulation close to the to-be-marketed commercial clinical drug product formulation.
- A single-dose rabbit tolerability study (report #s TDOC-0017265, 13-001) and a 3-month (Q4Wx3) GLP monkey ivt toxicology study (report # TDOC-0017689) were conducted with brolucizumab drug substance manufactured by (b) (4), to support Phase 3 and marketing.

6.1 Single-Dose Toxicity

Three non-GLP single-dose ivt toxicology studies were submitted to the BLA; they are not pivotal for safety. Monkey study # E-10-029 was submitted to support the original IND 112023 (as a follow-up study). Report # E-12-015 is was a single-dose rabbit ivt toxicology study to qualify increased levels of excipients, and report # 13-001 was a single-dose rabbit ivt tolerability study, to screen drug substance produce by (b) (4) prior to the monkey ivt toxicology study with the (b) (4) material (report # TDOC-0017689).

Report title	**Exploratory intravitreal injection study in I monkeys with a large-scale batch of ESBA 1008 (AL-86810) with increasing doses of endotoxin**	
Report #s	• TDOC-0011909 • E-10-29	
Summary	• The results of this study are not directly relevant to the to-be-marketed clinical drug product. • This non-GLP single-dose study was conducted as a follow-up to report # TDOC-0011462. • A single unilateral ivt dose of brolucizumab caused severe ocular inflammation. A no observed adverse effect level (NOAEL) was not identified. • Alcon attributed the inflammation to the high level of endotoxin in the batch (0.042 EU of endotoxin per mg of brolucizumab); the batch was not tested clinically	
	• Groups of 2 cynomolgus monkeys received a single unilateral dose of 0, 500, 1000, 2000, or 3000 µg/eye of brolucizumab. o The study was originally planned as an 8-day observational study, with return of animals to colony. • However, based on the retinal degeneration observed, additional endpoints were added, including necropsy on D50, to assess recoverability.	
Report	Report date	April 7, 2011

<table>
<tr><td rowspan="4">details</td><td>In-life period</td><td>• In-life phase initiated June 15, 2010 (i.e. initiating just before the end-of-in-life for report # TDOC-0011462, reviewed below)
• In-life phase completed August 3, 2010 (i.e. prior to the start of the follow-up non-GLP repeat-dose study, report # TDOC-0012900)</td></tr>
<tr><td>GLP status</td><td>No</td></tr>
<tr><td>Study laboratory</td><td>Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134</td></tr>
<tr><td>File location</td><td>BLA module 4.2.3.1 Single-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\pcs-rtdoc-0011909\pcs-rtdoc-0011909—pre-clinical-study-report.pdf)</td></tr>
<tr><td rowspan="5">Methods</td><td>Test article</td><td>Brolucizumab (AL-86810, ESBA1008), batch # ESBA1008-0949TR2 (numbered -10, -20, -40, and -60 for the 10, 20, 40, and 60 mg/ml test articles, respectively)</td></tr>
<tr><td>Vehicle formulation</td><td>• (b) (4)
• Review note: this was an early formulation, not the final to-be-marketed formulation. See Table 1 of this review above.</td></tr>
<tr><td>Monkey model</td><td>• A total of 10 cynomolgus monkeys (2 male, 8 female) were used
• Age range 2.9 to 5.8 years
• Weight range 2.2 to 4.3 kg</td></tr>
<tr><td>Doses:</td><td>Single intravitreal (ivt) 50 µl injection, right eye (OD) only
<table>
<tr><th>Brolucizumab dose (µg/eye)</th><th>Endotoxin (EU/eye)</th><th>Monkey sex</th></tr>
<tr><td>0 (buffer)</td><td>0</td><td>1 male, 1 female</td></tr>
<tr><td>500</td><td>0.021</td><td>2 females</td></tr>
<tr><td>1000</td><td>0.042</td><td>2 females</td></tr>
<tr><td>2000</td><td>0.084</td><td>2 females</td></tr>
<tr><td>3000</td><td>0.126</td><td>1 male, 1 female</td></tr>
</table></td></tr>
<tr><td>Originally-planned endpoints</td><td>• Twice daily checks for morbidity and mortality
• Clinical observations on D1 only (after dosing)
• Body weight: pre-dose and D8
• Ophthalmoscopy (slit lamp biomicroscopy, indirect ophthalmoscopy, intraocular pressure (IOP)): pre-dose, 24 and 48 hours post-dose, D8.
• Hematology and coagulation: pre-dose and D2</td></tr>
</table>

		• Ocular scoring using the Hackett and McDonald method[22].
	Additional endpoints	• Body weight on D46 and D50 • Ophthalmoscopy on D46 and D50 • Scotopic and photopic electroretinography (ERG) on D49. The report did not include a description of the ERG methodology, and this is a study limitation. • Hematology and coagulation on D49 • Necropsy on D50 o Organ weights limited to kidney, liver, spleen, ovary, and testes o Histopathology limited to the eye, ocular adnexa, and spleen
Results Results	Ocular endpoints	• All brolucizumab-treated eyes exhibited minimal aqueous flare at 24 and 48 hours post-dose, but not at D7 or D45. • One monkey at 500 µg/eye exhibited only moderate anterior chamber cells at 24 and 48 hours (recovered by D7 without veterinary treatment). • The other monkey at 500, and all monkeys ≥ 1000 µg/eye exhibited severe ocular inflammation: grade 4 anterior chamber cells at 24 and 48 hours post-dose. o The severity of inflammation reportedly increased (peaking at 24 hours through D3). All of these monkeys received topical antibiotics and steroids for ≥ 7 days; presumably this care reduced the duration of severe inflammation. • For the monkeys with severe ocular inflammation, a dose-response was apparent: o 500 µg/eye: anterior chamber cells were grade 1 (minimal severity) by D7, not observed at D45. o 1000 µg/eye: anterior chamber cell severity reduced to grade 1 by D7, not observed at D45. Minimal-to-mild anterior vitreous cells observed at all time points (i.e. to D45). Sluggish aqueous flow noted. o 2000 µg/eye: as above, moderate fibrin in the anterior chamber at 24 hours, one of the two monkeys had minimal anterior chamber cells persisting at D45.

[22] Hackett, R.B. and McDonald, T.O. (1996) “Ophthalmic Toxicology” and “Assessing Ocular Irritation” in Dermatotoxicology, 5th Edition, Eds. F.N. Marzulli and H.I. Maibach. Taylor and Francis Publishing Corp., Washington, D.C., pp. 299-306 and pp. 557-567.

		o 3000 µg/eye: as above, plus reduced light reflex and vitreal haze • ERG: o Authors conclude that no treatment-related effects were detected, and this reviewer concurs • Ocular histopathology: all of the brolucizumab-treated eyes exhibited minimal vitreal pigmented cell accumulation.
	Systemic endpoints	• No early mortality • The high-dose female (#5501) lost 4.8% of body weight from D-1 (pre-dose) to D8, and did not recover this weight by D50. The small group size precludes an assessment of treatment-relatedness. • No remarkable findings for hematology and coagulation. • Histopathology: both (2/2) spleens at 3000 µg/eye exhibited slight lymphoid depletion.

Report title	**An exploratory single dose intravitreal injection ocular toxicity study of various excipients ((b) (4) and sucrose) in New Zealand White rabbits with a four-week observation period**	
Report #s	• TDOC-0015618 • E-12-015	
Key findings	• The results do not raise concern for the brolucizumab drug product • This non-GLP single-dose ivt study did not test brolucizumab, and did not test the clinical vehicle formulation	
	• No test-article related toxicity was observed. This study qualifies a higher ivt dose of sucrose and sodium citrate than are used in the clinical formulation. • *Review note*: as noted above, this review does not document review of the rabbit ivt excipient repeat-dose study (report # TDOC-0015618). The formulations tested in this study are slightly different than were tested in the rabbit repeat-dose study.	
Report details	Report date	July 20, 2012
	Start of in-life	April 24, 2012
	GLP status	No
	Study laboratory	• Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134 • Ocular histopathology by (b) (4)
	File location	BLA module 4.2.3.1 Single-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-

		rep\423-tox\4231-single-dose-tox\pcs-rtdoc-0015618\pcs-rtdoc-0015618—pre-clinical-study-report.pdf)

Four formulations tested:

Descriptor	"AL-86810 ophthalmic solution vehicle"	(b) (4) in AL-86810 ophthalmic solution vehicle"	(b) (4) in AL-86810 ophthalmic solution vehicle"	(b) (4) sucrose + (b) (4) in AL-86810 ophthalmic solution vehicle"
Lot #	12-65926-1	12-65951-1	12-65928-1	12-66022-1
Group #	Group 1	Group 2	Group 3	Group 4
0	-	(b) (4)	-	-
(b) (4)				
Sucrose				
(b) (4)				
pH				
Osmolality (mOSM/kg)				
Endotoxin (EU/ml)				

Water for injection: q.s. to 100%. (b) (4)

"-" = not present in the formulation

Methods	Test species	3 male NZW rabbits/group
	Dosing	• Single unilateral (OD) ivt injection • 50 µl volume
	Endpoints:	• Twice daily checks for morbidity and mortality • Clinical observations made once weekly + the day of necropsy • Weekly body weight • Slit lamp biomicroscopy (with dilated lens evaluation): pre-dose, D2, 4, 8, 15, 22, 29 • Indirect ophthalmology: pre-dose and D29 only • IOP: pre-dose, D2, 7, 14, 28 • Necropsy: D30. Histopathology limited to the eye, optic nerve, and ocular adnexa

Results:	• No treatment-related deaths, clinical signs, or body weight changes were apparent. • Minimal conjunctival congestion was observed in all dose groups. This reviewer attributes this effect to the dosing procedure. • No treatment-related effects noted for other ophthalmoscopy endpoints. • No gross pathology findings were noted for any animal (ocular or systemic). • No remarkable ocular histopathology was observed .

Report title	**AL-86810 (ESBA1008, (b) (4) material): an acute intravitreal screening evaluation in New Zealand White rabbits**	
Report #	• TDOC-0017265 • 13-001	
Key findings	• The results of this study are not directly relevant to the to-be-marketed clinical drug product. • "This screening study was conducted to assess the potential effects of > 0.5 EU/mL endotoxin levels in the current drug product"	
	• 5 NZW rabbits received a single unilateral (OD) dose of 6 mg of brolucizumab (50 µl) • Treatment caused minimal conjunctival congestion and WBC in the posterior chamber at 48 hours post-dose (the only time point examined).	
Report details	Report date	November 6, 2013
	Date of dosing	September 3, 2013
	GLP status	No
	Study laboratory	Alcon Research Ltd. 6201 S. Freeway, Fort Worth, Texas 76134
	File location	BLA module 4.2.3.1 Single-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4231-single-dose-tox\pcs-rtdoc-0017265\pcs-rtdoc-0017265—pre-clinical-study-report.pdf)
Methods	Test article	• Brolucizumab (AL-86810-(b) (4) Material), 120 mg/ml, lot # 13-68149-1. • Review note: neither the formulation nor the amount of endotoxin was identified in the study report or elsewhere in the BLA. The BLA's Toxicology

		Tabulated Summary (BLA module 2.6.7)[23] explicitly notes that the drug substance purity and endotoxin levels were not specified.
	Single dose group:	• Two male and three female NZW rabbits were dosed once with 6 mg/eye OD. • *Review note*: no examination of the undosed contralateral eye (OS) was reported. No concurrent negative control group.
	Endpoints:	• Ocular examination was limited to OD only at D3 (approximately 48 hours after dosing): slit-lamp biomicroscopy, indirect ophthalmic examination, and vitreous white blood cell (WBC) count. • No other endpoints (disposition of animals not reported).
Results:	• All (5/5) eyes exhibited grade 1 conjunctival congestion • Two eyes (2/5) exhibited minimal anterior chamber inflammatory cells • WBC in the vitreous were detected for all animals; the mean was 62 cells (range 10 to 140 cells). • The authors considered the drug substance lot to be well tolerated.	

6.2 Repeat-Dose Toxicity

- Two pilot non-GLP repeat-dose ivt toxicology studies were conducted, in cynomolgus monkeys (report #s E-10-003 and E-10-043), and were submitted in the original IND. They are not pivotal to the safety of the BLA.
- Three pivotal, GLP repeat-dose toxicology studies were conducted, in the cynomolgus monkey, using the intravitreal route of administration.
    - N-10-104: Q3Wx3. Dosing began September 21, 2010. Submitted in the original IND.
    - N-12-042: Q4Wx6. Dosing began October 16, 2012.
    - N-14-007: Q4Wx3, testing the new drug substance. Dosing began May 6, 2014. The report was finalized October 31, 2014, and submitted to the IND on December 9, 2014.

Report title	**Exploratory dose range finding intravitreal injection study in monkeys with ESBA1008 (AL-86810)**
Report #s	• TDOC-0011462 • E-10-003

[23] BLA module 2.6.7 internally accessible from the EDR via: \\cdsesub1\evsprod\bla761125\0001\m2\26-nonclin-sum\toxicology-tabulated-summary.pdf

<table>
<tr><td>Key findings</td><td colspan="2">• The results of this study are not directly relevant to the to-be-marketed clinical drug product.
• Alcon attributed the inflammation to the high level of endotoxin in the batch (0.316 EU of endotoxin per mg of brolucizumab); the pre-GMP batch was not tested clinically.
• Study was not designed to identify an ocular or systemic NOAEL (not GLP, groups sizes were only 2 monkeys/dose)
o Dose-response for retinal degeneration observed for all brolucizumab-treated monkeys (i.e. ≥ 500 µg/eye)
o ERG B-wave amplitude changes ≥ 2000 µg/eye
o No systemic toxicity observed (up to the high-dose of 6000 µg/eye)
• Based on the in-life observations for this study, the single-dose non-GLP monkey study (report # TDOC-0011909) was initiated to follow-up on the cause of ocular inflammation and toxicity.</td></tr>
<tr><td rowspan="4">Report details</td><td>Report date</td><td>April 5, 1011</td></tr>
<tr><td>In-life initiated</td><td>March 30, 2010</td></tr>
<tr><td>GLP status</td><td>No</td></tr>
<tr><td>Study laboratory</td><td>Alcon Research Ltd.
6201 S. Freeway, Fort Worth, Texas 76134</td></tr>
<tr><td>File location</td><td>BLA module 4.2.3.2 Repeat-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\pcs-rtdoc-0011462\pcs-rtdoc-0011462—pre-clinical-study-report.pdf)</td></tr>
<tr><td rowspan="3">Method details</td><td>Test article</td><td>Brolucizumab (ESBA1008)
• For the (b) (4)% solution, the purity was 97.7%, endotoxin was 0.316 EU/ml, and host-cell DNA was 19.5 pg/ml</td></tr>
<tr><td>Formulation</td><td>• The “initial formulation” was tested: (b) (4)
• Review note: this is not the to-be-marketed commercial formulation (formulation B), or the formulation tested Phase 2/3 (formulation A).</td></tr>
<tr><td>Species</td><td>Cynomolgus monkeys (14 total)
• Age range 2 to 6 years at initiation
• Body weight 2 to 9 kg at initiation</td></tr>
<tr><td colspan="3">Dosing:
• Single eye (OD) ivt dosing on D1 and D36. Necropsy on D79 (i.e. 43 days after the second dose)
• Dose groups were 0, 500, 1000, 2000, 3000, 0, or 6000 µg/eye</td></tr>
</table>

Group #	# of monkeys	Ivt dose (µg/eye)	Ivt volume (µl/eye)

1	2 females	0	50
2	2 females	500	50
3	2 females	1000	50
4	2 females	2000	50
5	2 females	3000	50
6	2 males	0	100 [a]
7	2 males	6000	100 [a]

[a] For the 100 µl injections, dosing was preceded with aspiration of 75 µl from the OD vitreous, with the goal of accommodating the intraocular volume.

General endpoints: results	• No findings detected for clinical signs, body weight, intraocular pressure (IOP), pachymetry, clinical pathology (hematology, coagulation, clinical chemistry) • Biomicroscopy detected transient moderate ocular inflammation after dosing: moderate white blood cells (WBCs) in the anterior chamber, with minimal aqueous flare, resolving with 7 days post-injection • Indirect ophthalmoscopy (fundoscopy) failed to detect the retinal changes observed upon histopathology evaluation • No gross pathology findings	
Electro-retinography (ERG)	Methods	• Scotopic and photopic ERG, OD only: pre-study, D25, D73 • No method details (methodology can be inferred from the results presented): amplitude and time for rod B, mixed A, mixed B, and cone B
	Results	• The authors report: o For 2000 and 3000 µg/eye: ↓ rod-B wave amplitude, ↓ mixed B-wave amplitude, ↓ cone B-wave amplitude on D73 o For 6000 µg/eye: : ↓ rod-B wave amplitude, ↓ mixed B-wave amplitude, ↓ cone B-wave amplitude, and also ↓ mixed A-wave amplitude on D25 and D73 o ERG findings “correlated with the histopathological findings” in the retina.

Table 23: OD ERG amplitude results for the first repeat-dose ivt monkey toxicology study (report # TDOC-001462)

Treatment group	Study Days	Rod B Amp (µV)	Mixed A Amp (µV)	Mixed B Amp (µV)	Cone B Amp (µV)
1: Vehicle (50 µL/dose)	Pre	264.90	204.20	390.70	184.65
	25	232.75	149.90	322.55	144.15
	73	218.30	168.40	363.10	159.75
2: ESBA1008 (500 µg/dose)	Pre	204.25	163.60	308.35	101.45
	25	211.35	175.95	309.95	99.55
	73	147.85	166.10	247.45	84.20
3: ESBA1008 (1000 µg/dose)	Pre	198.70	157.50	305.60	103.85
	25	210.65	146.55	303.45	92.25
	73	164.50	155.80	270.10	81.60
4: ESBA1008 (2000 µg/dose)	Pre	179.45	114.00	262.25	94.95
	25	136.80	112.40	212.25	68.35
	73	**45.30**	136.50	**166.10**	**44.15**
5: ESBA1008 (3000 µg/dose)	Pre	188.20	99.25	256.35	122.60
	25	154.05	130.85	273.75	99.05
	73	**101.00**	113.50	**173.20**	**64.65**
6: Vehicle[b] (100 µL/dose)	Pre	178.70	102.00	270.40	122.00
	25	164.70	89.25	260.50	112.25
	73	193.25	134.15	259.65	122.95
7: ESBA1008[b] (6000 µg/dose)	Pre	207.85	174.30	304.15	123.60
	25	**144.30**	**129.35**	**238.65**	**89.30**
	73	**17.45**	**88.45**	**89.30**	**21.55**

Gross necropsy:

- Limited gross necropsy was conducted; results reported for the transponder, eyes, ocular adnexa, ovary, liver, gallbladder, kidney, uterus, oviduct, testes, trachea, lungs, bone, and optic nerve. Only the kidneys, liver, ovary, and testes were weighed.
- No gross pathology findings reported.

Histopathology:

- Limited histopathology: both eyes, optic nerve, lacrimal glands; knee-joint bone (the physes and epiphysis of the femur), kidneys, liver, lungs, oviduct, ovary, uterus, and testes. (page 24)
- The only remarkable findings occurred in the treated (OD) eye:

Table 24: Selected OD histopathology for the first repeat-dose ivt monkey toxicology study (report # TDOC-001462)

Finding	Severity	0	500 µg	1000 µg	2000 µg	3000 µg	0	6000 µg
# of females		2	2	2	2	2	0	0
# of males		0	0	0	0	0	2	2
Retinal degeneration	Minimal	0	2	2	0	0	0	0
	Mild	0	0	0	1	2	0	0
	Moderate	0	0	0	1	0	0	2
Vitreal cells	Minimal	0	0	2	2	2	1	1
	Mild	0	0	0	0	0	0	1

TK

- TK was analyzed using a quantitative ELISA method with a lower limit of quantitation (LLOQ) of 2.00 ng/ml for serum and 20.0 ng/ml for vitreous humor.
- Vitreous humor was collected at necropsy only (D79) for TK and ADA; no brolucizumab was detectable (LLOQ of 20.0 ng/ml)
- Blood was collected after the first injection (0, 6, 12 hours; D3, 8, 14, 29), and after the second injection (0, 6, 12 hours; D37, D38, D43, D50, D65, D79).
- For serum, accumulation was not apparent (comparing D36 and D1).

Table 25: Serum TK for the first repeat-dose ivt monkey toxicology study (report # TDOC-001462)

Dose	Day	C_{max} (ng/ml)	$AUC_{0\text{-}168h}$ (ng/ml*hr)
500 µg	1	58.9	2330
	36	35.0	1960
1000 µg	1	114	4130
	36	135	4240
2000 µg	1	198	13,000
	36	212	10,100
3000 µg	1	349	16,700
	36	283	13,800
6000 µg	1	583	24,300
	36	537	32,200

ADA

- Pre-dose ADA was detected in 4/14 animals.
- 5 monkeys developed specific antibodies (initially detected at D29)
- Vitreous humor ADA was detected in both monkeys treated with 6000 µg (but not in the lower dose groups)

Report title	**Exploratory ocular toxicity evaluation of repeat intermittent intravitreal injections (Q6Wx2) with various batches of AL-86810 (ESBA1008) in cynomolgus monkeys**	
Report #s	• TDOC-0012900 • E-10-043	
Key findings	• The purpose of this study was to further investigate the retinal degeneration observed previously (report # TDOC-0011462). • The ocular toxicity of the previous ("pilot lab batch") was verified. • The "GMP batch" caused severe ocular toxicity in 1/3 treated eyes	
Report details	Report date	April 7, 2011
	Start of in-life	October 19, 2010
	GLP status	No
	Study laboratory	Alcon Research Ltd. 6201 S. Freeway Fort Worth, Texas 76134
	File location	BLA module 4.2.3.2 Repeat-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\pcs-rtdoc-0012900\pcs-rtdoc-0012900—pre-clinical-study-report.pdf)
Methods	Species	Cynomolgus monkeys • Age range 2.9 to 4.5 years at study initiation • Body weight range 2.6 to 4.5 kg at study initiation • 3 monkeys/dose group (either 2 males and 1 female, or 2 females and 1 male)
	Dosing	• OD Ivt injection on D1 and D43, with necropsy on D86 (i.e. 6 week recovery). • Dose volume 50 µl for all groups. • 5 different vehicles (i.e. without brolucizumab) • 3000 µg brolucizumab, of the same "pilot lab batch" tested under report # TDOC-0011462. Lot # 100208MK001. • 3000 µg brolucizumab of a "GMP" batch. Lot # ESBA1008-101601-2-60.
Results for 3000 µg pilot lab batch	• All 3 treated eyes exhibited severe WBC in the anterior chamber (peak at 24-72 hours after the first injection); partial recovery beginning at D7) • OD ERG on D81 (scotopic and photopic): o One monkey (# 5502) with no B wave response o For the other two: ↓ rod B wave amplitude, ↓ mixed B wave amplitude, ↓ cone B wave amplitude. o All 3 treated eyes had moderate-to-severe retinal degeneration, minimal retinal detachment, and mild optic nerve degeneration	

Results for 3000 µg GMP batch	• One treated eye (# 7001) exhibited inflammation: o Severe inflammation peaking at 48 hours after the first dose: severe WBC in the anterior chamber; moderate conjunctival congestion, iritis, corneal cloudiness; minimal conjunctival swelling and flare; fibrin clot in the anterior chamber. o This animal received topical ocular antibiotic/steroid. Partial recovery by D36. o The second injection caused moderate WBC in the anterior chamber, minimal conjunctival congestion, and minimal aqueous flare. o Persistent low IOP • No effect on OD ERG at D81 apparent • For #7001, the right eye exhibited mild retinal degeneration, mild retinal pigmented cell accumulation, and mild vitreal fibrovascular proliferation

Study title: Ocular toxicity evaluation of repeat intermittent intravitreal injections (Q3Wx3) of AL-86810 (ESBA 1008) in cynomolgus monkeys with a 3-week post dose observation period

Study no.:	• TDOC-0012707 • N-10-104
Study report location:	BLA module 4.2.3.2 Repeat-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\pcs-rtdoc-0012707\pcs-rtdoc-0012707—pre-clinical-study-report.pdf)
Conducting laboratories and locations:	• In-life: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134 • Pathology: (b) (4) [redacted] • Pathology peer review: (b) (4) [redacted] • Bioanalytical report: (b) (4) [redacted] • Test article characterization: (b) (4) [redacted]
Report status and date:	Final, April 11, 2011
Date of study initiation:	September 16, 2010
In-life initiated:	September 21, 2010

GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	Brolucizumab (ESBA1008), manufactured under GMP by (b) (4) • Vehicle: lot # ESBA1008-101601-2-0 • 1% test article: ESBA1008-101601-2-10, purity 97.6% • 2% test article: ESBA1008-101601-2-20, purity 97.8% • 6% test article: ESBA1008-101601-2-60, purity ≥ 97.8% *Review notes*: o for all lots, endotoxin < 0.05 EU/ml o Vehicle and test articles were sterile (5 micron) filtered just prior to injection

Key Study Findings

- This GLP study used the (b) (4) drug substance in the "initial formulation", which was subsequently tested in Phase 1.
- This reviewer concludes that the study did not identify an ocular NOAEL or systemic NOAEL.
  - Severe ocular inflammation was observed sporadically, including the vehicle control group and at the low-dose (500 µg/eye).
  - An apparent dose response was observed for lymphoid hyperplasia (increased spleen weight, spleen and lymph node hyperplasia; reduced thymus lymphoid depletion), but the monkeys were infected (lung and GI), which confounds interpretation of the study. The response may be secondary to infection, rather than drug exposure.
  - One treated monkey exhibited broken teeth (teeth were not examined at necropsy). In the absence of tooth findings noted for the other studies, the relationship of this effect to treatment is unclear.
  - Three treated monkeys exhibited skeletal muscle degeneration/necrosis. The relationship of this effect to treatment is unclear.

Methods	
Doses:	0, 500, 1000, 3000, or 6000 µg/eye of brolucizumab (ESBA1008) OD
Frequency of dosing:	Once every 3 weeks x 3 (D1, 22, 43). Necropsy after a 3-week recovery period, on D64 (males) or D65 (females).
Route of administration:	OD ivt injection • Under anesthesia, the globe was immobilized (forceps or a stay suture

	through the conjunctiva and Tenon's capsule) • Prior to injection, ≤ 100 µl of vitreous was aspirated through a 25 to 27 gauge needle, with the goal of accommodating the dose. [Review note: • The ivt injection procedure used a 30 gauge needle, placed through the sclera 3 to 5 mm posterior to the temporal limbus, angled to avoid the lens, to target delivery to the inferior-temporal region of the eye.
Dose volume:	100 µl
Formulation/Vehicle:	(b) (4) sodium citrate, (b) (4) Review note: this is the "initial formulation", **not the to-be-marketed commercial formulation**.
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3/sex/dose
Age at start of dosing:	2 to 4 years
Weight at start of dosing:	2.2 to 4.4 kg
Satellite groups:	None
Deviation from study protocol:	Protocol deviations are documented on report page 59. This reviewer concludes that none of the deviations affected the outcome of the study or the interpretability of the results.

Observations and Results

Checks for Mortality and Morbidity; Clinical Signs; Body Weight, and Food Consumption

- Methods:
  - Animals were checked twice daily (morning and afternoon) for morbidity and mortality. The morning check included an evaluation of food consumption and fecal/urine output.
  - Cage-side observations were recorded weekly (in the morning).
  - Animals were removed from the cage for clinical observations once weekly (in the morning), and on the day of necropsy.
  - Body weights were recorded once weekly (with the once weekly removal from cage for evaluation of clinical signs)
  - [no scheduled veterinary physical examinations]
- The only remarkable result was "broken teeth" for a low-dose male (# 2001), noted on D36 and 39 (page 215). The observation of broken teeth was not documented for subsequent days (i.e. D42-64).
  - No further details regarding this observation were provided.

- Gross pathology did not include evaluation of teeth.

Ophthalmoscopy

- Methods:
  - Slit-lamp biomicroscopy: pre-dose; at 6, 48, 72 hours and 7 days after each injection, weekly for the remainder of the study, and not more than 2 days prior to necropsy on D64/65. The eyes were scored using the Hackett and McDonald scale.
  - Indirect ophthalmoscopy: pre-dose, 72 hours and 7 days after each injection, weekly for the remainder of the study, and not more than 2 days prior to necropsy on D64/65
  - Intraocular pressure (IOP): pre-dose, and weekly

- Results
  - The ivt dosing procedure caused transient inflammation: minimal-to-moderate WBC in the anterior chamber, minimal flare, pigmented and nonpigmented cells in the anterior chamber and anterior vitreous.
  - Treatment-related ocular inflammation was observed in the treated (OD) eye of 5 monkeys:

Female # 1502 (vehicle)	Moderate inflammation after the second dose. • Beginning on D36: severe corneal cloudiness, moderate WBC in the anterior chamber, minimal flare. • Veterinary intervention (topical ocular anti-inflammatory, anti-infective) from D36 toD43. • Inflammation largely resolved by D43; minimal WBC in the anterior chamber persisted.
Male #2002 500 µg/eye	Severe inflammation beginning on D24 (i.e. 2 days after the second ivt dose) through D63. • Moderate to severe areas of corneal cloudiness and white blood cells in the aqueous and no light reflex. • Minimal to mild flare. Severe fibrin from D24 to D29, resolving by D43. Grade ¾ cells in the aqueous • Grade 4 fibrin from D24-29, resolved by D43. • Veterinary intervention (topical ocular anti-inflammatory, anti-infective, and mydriatic agents) began on D29. Diagnosis of ocular infection made on D29; subcutaneous antibiotics administered for 14 days. • Severe vitreal haze on D50 and D57. • The 3rd ivt dose was administered on time (D43). • Notably, the TK analysis detected ADA, which apparently increased clearance of brolucizumab from plasma

Male # 3002 1000 µg/eye	Severe inflammation after the first dose: • Moderate-to-severe WBC in the anterior chamber, and minimal flare on D1-4 • Sluggish aqueous flow on D4 • Veterinary intervention (topical ocular anti-inflammatory, anti-infective, and mydriatic agents) from D4 to D8 • Minimal cells (pigmented and non-pigmented) in the anterior vitreous persisted
Female # 3502 1000 µg/eye	Severe inflammation after the first dose: • Severe WBC in the anterior chamber, minimal flare, and sluggish-to-no anterior chamber aqueous flow beginning on D3 • Beginning D4: no pupillary response to light, severe corneal surface cloudiness, severe fibrin in the anterior chamber, moderate flare, minimal conjunctival congestion and swelling, and iris bombe (i.e. swollen iris) • Veterinary intervention: topical ocular anti-inflammatory, anti-infective, and mydriatic agents from D4 to D15; topical ocular anti-inflammatory from D15 through D50. • Synechia was observed from D15-22. • Severe vitreal haze and severe cells in the vitreous beginning D8. • The 3rd ivt dose was administered on time (D43) • Inflammation partially resolved: o moderate vitreal haze from D29-necropsy o minimal WBC in the anterior chamber and cells (pigmented and non-pigmented) in the anterior vitreous persisted until necropsy
Female # 4502 3000 µg/eye	Moderate ocular inflammation after the last dose: • Mild inflammation beginning on D50 (mild WBC in the anterior chamber, minimal flare) • D53: severity increased (adding minimal fibrin in the anterior chamber, minimal conjunctival congestion, minimal flare, few cells in the anterior vitreous). • Veterinary intervention (topical ocular anti-inflammatory and anti-infective from D53 to necropsy • Minimal vitreal haze noted on D57. • Corneal cloudiness on D77 and 63 prevented evaluation of the lens.

o Note: the laboratory stopped IOP measurements for these 4 monkeys, to avoid worsening ocular inflammation (report page 40)

Electroretinography (ERG)

- ERG for both eyes (OU):evaluated during the last week prior to necropsy (i.e. no pre-dose ERG)
- Male #2002 (discussed above) exhibited severe ocular inflammation; his right eye did not respond to the light stimulation of the ERG.

Hematology, Coagulation, Clinical Chemistry

- No treatment-related effects were apparent for hematology, coagulation, or clinical chemistry.
- After overnight fasting, venous blood was collected for hematology, coagulation ,and clinical chemistry endpoints: pre-dose, D22 (the day after the second injection), and D63 (1 day prior to necropsy for males, 2 days prior to necropsy for females)
- No urine was collected for urinalysis.

Gross Pathology

- Gross pathology detected no treatment-related effects

Organ Weights

- A limited battery of organ weights was measured: adrenals, brain, heart, kidney, liver, ovary, spleen, and testes.
- Spleen weight was increased for treated females, but not treated males.

Table 26: male spleen weights for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Parameter	0	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
D64 body weight (g)	3610 ± 776	2969 ± 180	3309 ± 603	3065 ± 355	3102 ± 684
Spleen wt (g)	4.33 ± 1.51	2.84 ± 0.47 (-34%)	5.08 ± 1.41 (+17%)	3.23 ± 0.74 (-25%)	2.45 ± 0.67 (-56%)
Spleen:body wt	0.123 ± 0.051	0.093 ± 0.021 (-24%)	0.153 ± 0.021 (+24%)	0.107 ± 0.025 (-13%)	0.08 ± 0.017 (-34%)

Results presented as mean ± standard deviation

Table 27: female spleen weights for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Parameter	0	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
D65 body weight (g)	3164 ± 668	3865 ± 348	2799 ± 277	2447 ± 480	2853 ± 201

Spleen wt (g)	2.70 ± 0.87	3.79 ± 0.94 (+47%)	2.42 ± 0.32 (-10%	3.78 ± 1.74 (+39%)	4.06 ± 0.77 (+49%)
Spleen:body wt	0.090 ± 0.030	0.133 ± 0.035 (+47%)	0.087 ± 0.012 (-3%)	0.137 ± 0.049 (+52%)	0.147 ± 0.038 (+63%)

Results presented as mean ± standard deviation

Histopathology

After gross necropsy, tissues were fixed and preserved, and shipped to (b) (4) . Histopathology was performed by Dr. (b) (4)

Adequate Battery: Yes. Full systemic histopathology was evaluated. For the left and right eyes, line-listed results were reported for the anterior chamber, choroid, ciliary body, cornea, eye injection sites, eyelid, iris, lacrimal gland, lens, nasal turbinate, nasolacrimal duct, optic nerve, retina, sclera, and vitreous.

Peer Review: Partial (limited to the spleen and thymus; but not including the eye), by Dr. (b) (4)

Histological Findings

- No findings clearly related to brolucizumab were apparent.
- "Minor" eye trauma attributed to the pre-dose aspiration was observed in most OD eyes, including: minimal pigmented cells in the vitreous, minimal vitreal hemorrhage, minimal vitreal fibrovascular proliferation, and minimal retinal pigmented cells.
- The animals with in-life OD inflammation (discussed above) exhibited histopathology changes (e.g. vitreal chronic inflammation, vitreal mononuclear cell infiltrates, vitreal pigmented cells)
- All monkeys had lung findings (foci of macrophages in the alveolar septa and fibrous stroma of larger vessels and airways), attributed to lung mite infection (*Pneumonyssus spp.*). Many monkeys also have GI tract infection (protozoa or nematodes).
- Splenic (lymphoid depletion) and thymus (decreased thymic lymphoid depletion) effects were observed in treated monkeys. However, the presence of infections confounds the study. P/T cannot discern which effects may be responses to infection, and which might be due to brolucizumab.
    - Lymphoid depletion of the spleen was observed ≥ 1000 µg, with a dose response for incidence and severity. The authors did not consider the effect adverse (page 52). "*Splenic lymphoid depletion in all cases was characterized by lymphoid hypocellularity of the marginal zones, and periarteriolar lymphoid sheath (PALS) and few germinal centers, resulting in indistinct demarcation of the marginal zones from the PALS and overall decreased diameter of white pulp zones*."
    - The authors considered the thymus effect consistent with normal physiological involution (page 53)

- Two males (500 and 1000 µg) and one female (3000 µg) had skeletal muscle myofiber degeneration/necrosis. The report did not specify the muscle sectioned.

Table 28: Selected male histopathology for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Effect	Severity	0 (vehicle)	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
# of male monkeys examined		3	3	3	3	3
OD Retinal degeneration + detachment	Minimal to mild	0/3	0/3	0/3	0/3	0/3
	Moderate to severe	1/3	1/3	0/3	0/3	0/3
Spleen: lymphoid depletion	Combined incidence	0/3	0/3	0/3	1/3	2/3
	Minimal	0/3	0/3	0/3	1/3	1/3
	Mild	0/3	0/3	0/3	0/3	1/3
Spleen: hyperplasia	Combined incidence	3/3	2/3	3/3	1/3	1/3
	Minimal	1/3	2/3	0/3	0/3	1/3
	Mild	1/3	0/3	3/3	1/3	0/3
	Moderate	1/3	0/3	0/3	0/3	0/3
Mesenteric lymph node: lymphoid hyperplasia	Combined incidence	0/3	1/3	1/3	1/3	0/3
	Minimal	0/3	0/3	1/3	0/3	0/3
	mild	0/3	1/3	0/3	0/3	0/3
	moderate	0/3	0/3	0/3	1/3	0/3
Sub-mandibular lymph node: lymphoid hyperplasia	Mild	0/3	1/3	0/3	1/3	0/3
Thymus involution (lymphoid depletion)	Combined incidence	2/3	2/3	1/3	1/3	3/3
	Minimal	0/3	1/3	0/3	1/3	1/3
	Mild	0/3	1/3	1/3	0/3	1/3
	Moderate	1/3	0/3	0/3	0/3	0/3
	Severe	1/3	0/3	0/3	0/3	0/3
Pancreas: ectopic spleen	Present	0/3	0/3	1/3	0/3	0/3
Heart: chronic myocardium inflammation	Minimal	1/3	0/3	1/3	0/3	2/3
Skeletal muscle: myofiber degeneration / necrosis	Minimal	0/3	0/3	1/3	0/3	0/3
	Moderate	0/3	1/3	0/3	0/3	0/3
Lung – pigment deposition	Minimal to moderate	3/3	3/3	3/3	3/3	3/3

Lung – alveolus edema	Minimal to severe	2/3	3/3	2/3	2/3	3/3
Stomach: protozoan parasite	Present	1/3	0/3	0/3	0/3	0/3
Cecum: protozoan parasite	Present	2/3	0/3	3/3	1/3	1/3
Cecum: nematode or nematode ovum	Present	1/3	0/3	1/3	1/3	1/3
Colon: protozoan parasite	Present	1/3	0/3	0/3	0/3	0/3
Rectum: protozoan parasite	Present	1/3	0/3	0/3	1/3	0/3

Table 29: Selected female histopathology for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Effect	Severity	0 (vehicle)	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
# of female monkeys examined		3	3	3	3	3
OD cataract	Minimal	0/3	0/3	0/3	0/3	0/3
OD Retinal degeneration + detachment	Minimal to mild	1/3	0/3	0/3	1/3	0/3
	Moderate to severe	0/3	0/3	0/3	0/3	0/3
Spleen: lymphoid depletion	Minimal	0/3	0/3	1/3	0/3	0/3
	Mild	0/3	0/3	0/3	0/3	1/3
Spleen: hyperplasia	Minimal	2/3	1/3	0/3	0/3	1/3
	Mild	0/3	2/3	0/3	2/3	0/3
Submandibular lymph node: lymphoid hyperplasia	Minimal	1/3	0/3	0/2	0/3	1/3
	Mild	0/3	0/3	1/3	1/3	0/3
Thymus involution (lymphoid depletion)	Combined incidence	3/3	2/3	3/3	3/3	3/3
	Minimal	1/3	1/3	0/3	0/3	1/3
	Mild	1/3	0/3	2/3	1/3	2/3
	Moderate	0/3	0/3	1/3	2/3	0/3
	Severe	1/3	1/3	0/3	0/3	0/3
Heart: chronic myocardium inflammation	Minimal	2/3	1/3	2/3	0/3	1/3

Heart: chronic active epicardium inflammation	Mild	0/3	0/3	1/3	0/3	0/3
Skeletal muscle: myofiber degeneration / necrosis	Minimal	0/3	0/3	0/3	1/3	0/3
Lung – pigment deposition	Minimal to mild	3/3	3/3	3/3	3/3	3/3
Cecum: nematode	Present	0/3	0/3	1/3	0/3	0/3
Colon: protozoan parasite	Present	1/3	0/3	0/3	0/3	0/3
Rectum: protozoan parasite	Present	1/3	0/3	0/3	0/3	0/3

- Retinal degeneration/detachment:
    - Control male #1001: severe detachment, moderate degeneration, severe chronic active inflammation (page 642)
    - Control female # 1501: minimal detachment and degeneration (page 642)
    - 500 µg: one male (# 2002): severe detachment, severe degeneration, severe subretinal exudate, mild mononuclear cell infiltrate and pigmented cells in the retina (page 645)
    - 1000 µg: no retinas affected
    - 3000 µg: one female (# 4502) with minimal detachment, mild detachment, minimal subretinal exudate, and moderate mononuclear cell infiltrate
- The OD lens cataract was in high-dose female # 5503 (page 648)
- The treatment-relationship of the lymphoid hyperplasia (spleen, lymph nodes) is unclear, because this study was confounded by infection of the lung and GI tract.
    - Thymus involution (lymphoid depletion) may have been inhibited by treatment in males (clear dose-response for reduced incidence and severity) and females (dose response for reduced severity). This reviewer considers the decrease in thymic lymphoid depletion to be consistent with the observed splenic and lymph node hyperplasia.

Toxicokinetics

- Blood samples were collected on D1, 2, 3, 8, 21, 22, 23, 24, 29, 42, 43, 44, 45, 50, and 64 from all monkeys for TK and ADA. This corresponds to: 0, 6, 24, 48, and 168 hours after the each injection, and also 504 hours after the last injection.
    - *Review note*: the 504-hour (21 days) timepoint for TK and ADA was added as a protocol amendment on 12/14/2010 (pages 63-64), after the initiation of TK analysis of the serum samples on 12/08/2010 (page 395). Notably,

the in-life phase was completed 11/24/2010 (page 12) – meaning that the collection of samples was not under GLP. Because all of the 504-hour TK data points were below the LLOQ (2.00 ng/ml), this is a minor study discrepancy.

- Vitreous humor was collected at necropsy (approximately 100 to 150 µl samples) from the dosed (OD) eye only.
- Brolucizumab TK was measured using a quantitative ELISA, with a lower limit of quantitation (LLOQ) of 2.00 ng/ml for serum[24] and 20.0 ng/ml for vitreous humor[25].
- Serum C_{max} showed dose-proportionality for D1 and D22, but less-than-dose proportionality for D43.
- The authors noted (page 380) that one monkey (# 2002) from the 500 µg group had a rapid clearance of brolucizumab after the 3rd injection, which they attributed to ADA.
- For vitreous humor, the authors considered the 500, 1000, and 3000 µg/eye doses to show dose-proportionality, but considered the difference between 3000 and 6000 µg/eye to be more than dose proportional (page 379), suggesting saturation of clearance > 3000 µg from the eye. However, the within-group variability (reflected in the standard deviation calculation) precludes a clear determination of whether ocular clearance was actually saturated.

Table 30: Serum TK for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Day	Parameter	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
1	C_{max} (ng/ml)	28.3 ± 26.9	125 ± 120	107 ± 50.5	312 ± 170
	$AUC_{0\text{-}168h}$ (ng*h/ml)	1530 ± 1580	7830 ± 9490	701 ± 2060	20,700 ± 7870
22	C_{max} (ng/ml)	23.3 ± 13.4	98.5 ± 86.5	150 ± 32.5	295 ± 93.8
	$AUC_{0\text{-}168h}$ (ng*h/ml)	1760 ± 1170	6590 ± 5230	12,600 ± 4510	22,600 ± 5730
43	C_{max} (ng/ml)	40.5 ± 33.9	75.8 ± 47.4	177 ± 156	286 ± 91.8
	$AUC_{0\text{-}168h}$ (ng*h/ml)	1340 ± 1100	5160 ± 2670	13,900 ± 11,100	21,300 ± 4180

Results are presented as mean ± standard deviation for both sexes combined.

[24] The validation of the ELISA method for determination of brolucizumab in monkey serum was submitted to the BLA in report # TDOC-0013274 (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4221-analyt-met-val\dmpk-rtdoc-0013274\dmpk-rtdoc-0013274--pre-clinical-study-report.pdf)

[25] The validation of the ELISA method for determination of ADA against brolucizumab in monkey serum was submitted to the BLA in report # DTOC-0013305 (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\422-pk\4221-analyt-met-val\dmpk-rtdoc-0013305\dmpk-rtdoc-0013305--pre-clinical-study-report.pdf)

Table 31: Vitreous humor concentrations at necropsy for the TK for the first GLP monkey ivt (Q3Wx3) toxicology study (report # TDOC-0012707)

Day	Parameter	500 µg/eye	1000 µg/eye	3000 µg/eye	6000 µg/eye
64/65	Vitreous humor concentration (ng/ml)	99.6 ± 101	394 ± 446	682 ± 442	3160 ± 1670

Results are presented as mean ± standard deviation for both sexes combined

Anti-drug antibody (ADA) analysis

- Overall, ADA did not confound interpretation of the study results.
- A semi-quantitative ELISA method was used to detect ADA.
- Serum ADA:
  - Pre-dose ADA was observed for 1/6 controls (#1002), 1/6 monkeys at 500 µg (#2502), 3/6 monkeys at 1000 µg (all 3 females: # 3501, 3502, 3503), 4/6 monkeys at 3000 µg (all 3 males: # 4001, 4002, 4003; and one female,# 4501), and 2/6 monkeys at 6000 µg (# 5003, 5503).
  - Generally, ADA titers increased over time
  - None of the controls or high-dose (6000 µg) developed new ADA.
  - Two monkeys at 500 µg exhibited ADA beginning D22.
  - Two monkeys at 1000 µg exhibited ADA after D43
  - At 3000 µg, one monkey developed ADA at D22, and the other monkey was negative for ADA.
- Vitreous humor ADA: two monkeys (# 2002 in the 500 µg group, and # 4503 in the 3000 µg group) had ADA-positive vitreous humor (collected at necropsy); both also had serum ADA.
- A link between ADA and decreased systemic TK was apparent for only one monkey across studies. For the Q3Wx3 study (report # TDOC-0012707), one male at 500 µg/eye (# 2002), which exhibited severe ocular inflammation.

Dosing Solution Analysis

- The dosing solution analysis was adequate; no concerns were identified.
- Test article characterization was performed by the manufacturer of the drug product, (b) (4). Samples were analyzed for identity, protein content, purity, sterility, and endotoxin.
  - Test article was 98% pure by AIEX-HPLC, and 92% potent (by ELISA)
  - Two protein impurities were detected by reduced SDS-PAGE; one at 21 kDa and another at 2.5 kDa [brolucizumab is 26 kDa]; these were not quantified [report page 115]
  - Endotoxin tested < 0.05 EU/ml
  - DNA content < 1.6 pg/ml
  - Protein quantitation (after the end of dosing) found:
    - 10 mg/ml solution: 94% of nominal
    - 20 mg/mg solution: 95% of nominal
    - 60 mg/ml solution: 104% of nominal

Study title: AL-86810 (ESBA 1008): a six-month intermittent dose (Q4Wx6) intravitreal toxicity study in cynomolgus monkeys with a 3-month interim evaluation

Study no.:
- TDOC-0016684
- N-12-042

Study report location: BLA module 4.2.3.2 Repeat-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\pcs-rtdoc-0016684\pcs-rtdoc-0016684—pre-clinical-study-report.pdf)

Conducting laboratories and locations:
- In-life: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134
- Histopathology: (b) (4)
- Bioanalysis (brolucizumab and ADA): (b) (4)

Report status and date: Final, dated September 13, 2013

Date of study initiation: September 26, 2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Brolucizumab (AL-86810):

Group #	Test article	Lot #	Purity
1	Vehicle, old formulation	12-67014-1	-
2	Vehicle, new formulation	12-66760-1	-
3	2%, new formulation	12-66762-1	≥ 96%
4	6%, new formulation	12-66763-1	≥ 96.5%
5	12%, new formulation	12-66764-1	≥ 97%
6	6%, old formulation	12-67016-1	≥ 98%

Review note: Vehicle and test articles were sterile (5 micron) filtered just prior to injection.

Key Study Findings

- Groups of 3/sex cynomolgus monkeys were dosed in the right eye (OD) once per month x 6 with 0, 1, 3, or 6 mg/eye of brolucizumab, and were necropsied 4 weeks after the last dose. Two formulations were compared (at 0 and 6 mg/eye).
- The high-dose, 6 mg/eye/month, is the ocular no observed adverse effect level (NOAEL) for both formulations. Chronic ivt dosing with brolucizumab was associated with transient minimal ocular inflammation after dosing, and minimal histopathological changes at the end of in-life (conjunctival lymphoid hyperplasia, lymphocyte infiltration of the choroid and sclera, vitreal cells, and corneal vessels).
- The systemic NOAEL is 6 mg/eye.
    - Two high-dose (6 mg/eye) male monkeys exhibited "possible inguinal hernia" at veterinary examination. However, no abnormal findings were noted at gross necropsy. Therefore, the finding at examination is not clearly adverse. The relationship to treatment is unclear.
    - A dose-response for increased spleen weight was observed at 3 and 6 mg/eye. Using a 2-fold increase in spleen weight as a benchmark for toxicity, the 3 mg/eye dose was not adverse, and the 6 mg/eye dose was adverse for 1/12 treated monkeys.

- The results of this study support chronic clinical dosing with brolucizumab in the new formulation (i.e. tested Phase 2 and Phase 3). The study design included six groups:
    - The old vehicle [redacted] was tested at 0 and 6 mg/eye of brolucizumab (i.e. control and high-dose). The volume administered was [redacted] µl/eye.
    - The new vehicle [redacted] sucrose, [redacted] polysorbate 80, pH [redacted]) was tested at 0, 1, 3, or 6 mg/eye. Review notes:
        - The new vehicle is close to, but not exactly the same as, the to-be-marketed clinical formulation.
        - The Pharmacokinetic Written Summary (BLA module 2.6.4[26]) that the same formulation was tested in the Phase 2 clinical trial C-12-006 (OSPREY). The Drug Product Pharmaceutical Development – Clinical Trial Formulae (BLA module 3.2.P.2)[27] notes that the trials OWL, STRIKE, HAWK and HARRIER also tested this formulation.

[26] Module accessed via: \\cdsesub1\evsprod\bla761125\0001\m2\26-nonclin-sum\pharmacol-written-summary.pdf
[27] Accessed via: \\cdsesub1\evsprod\bla761125\0001\m3\32-body-data\32p-drug-prod\rth258-sol-for-inj\32p2-pharm-dev\pharmaceutical-development-clin-trial-formulae.pdf

Methods

Vehicles:
- "Control vehicle": (b) (4) q.s. with water for injection
- "Test vehicle": (b) (4) sucrose, (b) (4) polysorbate 80, (b) (4) q.s. with water for injection

Doses and dose volume: 0, 0, 1, 3, 6, 6 mg/eye

Group #	Dose	Volume
1	0	(b) (4) µl (old formulation)
2	0	50 µl (new formulation)
3	1 mg/eye	50 µl
4	3 mg/eye	50 µl
5	6 mg/eye	50 µl
6	6 mg/eye	(b) (4) µl (old formulation)

Frequency of dosing: Right eye (OD) only, once every 4 weeks x 6 (D1, 29, 56, 85, 113, and 141). Monkeys were sacrificed 4 weeks after the last dose (D170 for males, D171 for females)

Route of administration: Intravitreal (ivt) injection
- Under anesthesia, the globe was immobilized (forceps or a stay suture through the conjunctiva and Tenon's capsule)
- "*A 30-gauge needle attached to a sterile syringe, containing the vehicle or test article will be passed through the sclera 3 to 5 mm posterior to the temporal limbus and angled posterior to avoid the lens. The target location of the injection is the inferior-temporal region.*"

Species/Strain: Cynomolgus monkey (*Macaca fascicularis*)

Number/Sex/Group: 3/sex/group

Age at start of dosing: 4.5 to 5.5 years

Weight at start of dosing: 3.2 to 6.2 kg

Satellite groups:	None
Deviation from study protocol:	Study deviations are reported in Appendix A; the authors concluded, and this reviewer concurs, that the deviations did not affect the overall outcome or interpretability of the study results

Observations and Results

Checks for Mortality and Morbidity; Clinical Signs; Body Weight, and Food Consumption

- Methods:
  - Animals were checked twice daily (morning and afternoon) for morbidity and mortality. The morning check included an evaluation of food consumption and fecal/urine output.
  - Cage-side observations were recorded weekly (in the afternoon).
  - Animals were removed from the cage for clinical observations once weekly (in the afternoon), and on the day of necropsy.
  - Body weights were recorded once weekly (with the once weekly removal from cage for evaluation of clinical signs).
  - Veterinary physical examination (observations + vital signs) were performed pre-dose, on D113 (approximately 6 hours post-injection), and prior to sacrifice (D161-162).

Inguinal hernia

- Veterinary examination on D161 by [redacted] (b) (4) Alcon Research, Ltd] detected "possible inguinal hernia" for two males (2/6) dosed with 6 mg/eye (# 5001 in group 5, dosed with the new formulation; and # 6003 in group 6, dosed with the old formulation) [report pages 594 and 806].
  - For male # 5001, the veterinary comment was "Raised area, Abdomen: possible right inguinal hernia" (page 594)
  - For male # 6003, the veterinary comment was "Raised area, Abdomen = possible left inguinal hernia" (page 594)
- The study report did not document investigation of these possible hernias at necropsy (i.e. under gross necropsy); this is a serious study limitation. The report's description (methods and protocol) of the gross pathology procedure is deficient – the report does not use explicit language to the effect of "careful examination of the external surface of the body, all orifices, and the cranial,

thoracic and abdominal cavities and their contents."[28],[29] The gross pathology results did not include any listings corresponding to the scrotum/inguinal area.

- For the two inguinal hernias, the study director (page 31) and the veterinarian performing the physical examination (page 806) noted "this finding is a common developmental abnormality". The report did not provide a historical control incidence for the laboratory. The authors' remark suggests that relevant historical control data for the study laboratory should be readily available.
- A literature search by this reviewer found multiple mentions of inguinal hernia in cynomolgus and rhesus monkeys, but no monkey incidence data.

- For both males (#5001 and #6003), testes were immature.
- An information request was sent to the Applicant on 7/31/2019, and a response was received on 8/12/2019 (SD # 26).
  - The Applicant provided the laboratory's standard operating procedures for necropsy of primates[30].
    - The gross pathology procedure is adequate to detect gross abnormalities of the inguinal region.
    - The study laboratory used the (b) (4) data collection system. The Applicant verified that the study personnel would have recorded notable findings; the lack of recorded findings indicates that no findings were detected. This approach is acceptable.
    - Histopathology was not performed to attempt confirmation of the examination finding; this is a minor study limitation.
    - The study laboratory did not have relevant historical control data for inguinal hernia.
  - In support of the conclusion that the observations were non-adverse, the Applicant provided a book chapter by Cline et a. 2012[31]. The authors report for monkeys that:
    - Inspection for inguinal hernia is a routine part of physical examinations

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[28] OECD Test No. 452: Chronic Toxicity Studies. Adopted June 25, 2018. Accessed via https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

[29] Redbook 2000: IV.C.4.b Subchronic toxicity studies with non-rodents. 11/2003. Final guidance document. Center for Food Safety and Applied Nutrition (CFSAN), FDA. Accessed via: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/redbook-2000-ivc4b-subchronic-toxicity-studies-non-rodents

[30] Document # PROC-005381. Accessed via: \\cdsesub1\evsprod\bla761125\0020\m1\us\fda-response-nonclinical-proc-0005381.pdf

[31] Cline JM, Brignolo L, Ford E. 2012. Chapter 10: Urogenital System. In Nonhuman Primates in Biomedical Research (Abee, C. R., Mansfield, K., Tardif, S. D., Morris, T., eds.), 2nd ed, pp. 483–562.. Accessed via: \\cdsesub1\evsprod\bla761125\0020\m4\43-lit-ref\cline-2012p483.pdf

- For macaques, "the testes normally descend through the inguinal canal at birth, but remain near the opening of the canal until puberty. With sexual maturation at approximately 4 years of age, the testicles begin to increase in size"
- Inguinal hernia is a "common" condition of older overweight macaques. "Inguinal hernias are usually of no consequence" for nonhuman primates.
- Based on the Applicant's response, this review concludes that the inguinal observations were not clearly adverse. The relationship to treatment is unclear; the observations may be related to the monkeys being sexually immature.

Ophthalmoscopy

- Methods:
  - Slit-lamp biomicroscopy to evaluate the conjunctiva, cornea, anterior chamber, light reflex, lens, and iris of both eyes; scored using the Hackett and McDonald scale. Assessed the day prior to each injection; the first, third, and seventh day following each injection; and on D169 (prior to necropsy)
  - Indirect ophthalmoscopy evaluated the fundus for "optic nerve head characteristics, fundic vascular pattern (retinal and choroidal), and pigmentation/coloration characteristics" for both eyes. Intraocular pressure (IOP) was measured on the same days: on the day prior to each injection; the third day after each injection, and on D169 (prior to necropsy)
- Results: the injection procedure caused transient minimal inflammation. No treatment-related ophthalmoscopic effects were detected.

Electroretinography (ERG)

- No treatment-related ERG effects were apparent.
- For the right eye (OD) only, photopic and scotopic ERG were performed prior to dosing, and approximately 1 week prior to necropsy (D163-165). After dark adaptation, amplitude and peak time of the following waveforms were assessed:
  - Rod B wave (dark-adapted rod response)
  - Mixed A wave and mixed B wave (dark-adapted mixed rod-cone response)
  - Cone B wave (light-adapted cone-response)

Electrocardiogram (ECG)

- No treatment-related ECG effects were apparent.
- Performed pre-dose, on D113 (approximately 6 hours prior to the 5th injection), and prior to necropsy (D161-162).

- *Review note*: the timing of each endpoint did not cover the systemic T_{max} following intravitreal dosing. These data are of limited utility to support safety.

Hematology, Coagulation, Clinical Chemistry

- No treatment-related effects were apparent for hematology, coagulation, or clinical chemistry.
- After overnight fasting, venous blood was collected for hematology, coagulation, and clinical chemistry endpoints: pre-dose, D80 (prior to the 4th injection on D85), and D168 (prior to necropsy)
- No urine was collected for urinalysis.

Gross Pathology

- Males were sacrificed on D170; females were sacrificed on D171.
- No treatment-related effects on gross pathology were apparent.

Organ Weights

One monkey at 6 mg/eye (# 5001) exhibited a 2-fold increase in spleen weight compared to controls (without a concomitant change in hematology parameters, gross pathology, or histopathology).

- At sacrifice, weights were collected for a limited panel of organs: adrenal glands, brain, heart, kidney, liver, ovary, spleen, and testes.
- Spleen weights were higher for treated animals compared to controls.
    - Males:
        - For the old formulation (i.e. comparing groups 1 and 6), the 6 mg/eye dose increased absolute spleen size by +26%, and +32.8% by spleen:body weight.
        - For the new formulation (i.e. comparing groups 2-5), the increase in absolute spleen weight was + 3.7%, +19.6%, and +26.6% for the 1, 3, and 6 mg/eye groups respectively.
    - Females: the increase in spleen weight was only apparent for group 5 (6 mg/eye, new formulation): + 26% (absolute weight), + 13% (spleen:body weight).
    - Not a clear difference between the formulations for increased spleen weight.
    - The authors noted that male # 5001 (in group 5) had a remarkably high spleen weight: absolute weight of 9.30 g and relative weight of 0.16 (both parameters represent a 2-fold increase over vehicle control).
    - Notably, review of the hematology data did not detect a corresponding change in WBC counts (for monkey # 5003, or across groups).
- The authors noted (page 47) that systemic bevacizumab was associated with increased spleen weight at ≥ 50 mg/kg (species not specified).
- The adrenal weight data are variable between the two control groups; no treatment-related effect is clearly apparent. The results suggest a possible slight increase with increasing dose.

Table 32: Selected male organ weights: 6-month ivt monkey toxicology study (report # TDOC-0016684)

Parameter (D170 necropsy)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	0 (old formulation)	0 (new formulation)	1 mg/eye	3 mg/eye	6 mg/eye	6 mg/eye (old formulation)
Mean body weight (g)	5844 ± 613	6056 ± 1513	5935 ± 645	5574 ± 1163	5890 ± 900	5780 ± 674
Spleen (g)	4.203	4.657 (100%)	4.833 (104%)	5.573 (120%)	5.897 (127%)	5.320 (126%)[a]
Spleen:body weight	0.070	0.080 (100%)	0.080 (100%)	0.07 (88%)	0.103 (129%)	0.093 (132%)[a]
Spleen:brain	5.853	6.757	6.890	7.463	8.127	7.630
Adrenals (g)	0.773 ± 0.051	0.453 ± 0.124	0.627 ± 0.245	0.619 ± 0.109	0.470 ± 0.046	0.533 ± 0.039
Adrenal:body weight	0.0133	0.0080	0.0110	0.0113	0.0083	0.0090
Adrenal:brain	1.103	0.657	0.857	0.823	0.663	0.767

[a] Compared to group 1. Results presented as mean ± standard deviation.

Table 33: Selected female organ weights: 6-month ivt monkey toxicology study (report # TDOC-0016684)

Parameter (D170 necropsy)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	0 (old formulation)	0 (new formulation)	1 mg/eye	3 mg/eye	6 mg/eye	6 mg/eye (old formulation)
Mean body weight (g)	4266 ± 235	4457 ± 1395	3855 ± 610	3742 ± 219	3387 ± 316	4104 ± 351
Spleen (g)	4.227	3.503 (100%)	3.547 (101%)	3.427 (97%)	4.443 (126%)	2.927 (69%) [a]
Spleen:body weight	0.103	0.080 (100%)	0.093 (116%)	0.093 (116%)	0.117 (146%)	0.070 (67%) [a]
Spleen:brain	6.200	5.150	5.000	5.767	6.737	4.323
Adrenals (g)	0.410 ± 0.111	0.542 ± 0.070	0.448 ± 0.058	0.432 ± 0.215	0.498 ± 0.033	0.600 ± 0.076
Adrenal:body weight	0.0097	0.0127	0.0117	0.0113	0.0130	0.0147
Adrenal:brain	0.603	0.803	0.640	0.730	0.753	0.917

[a] Compared to group 1. Results presented as mean ± standard deviation.

Histopathology

Notes regarding the adequacy of the histopathology battery:

- The study pathologist was Dr. (b) (4)
- A full systemic battery of tissues was evaluated, including the eye eyes, optic nerves, eyelids, lacrimal glands, and nasal lacrimal regions.
- *The protocol did not pre-specify ensuring that sections through the macula/fovea were collected for evaluation. This is a study limitation.*
- For the eyes: "*At minimum, nine sections (slides) from each eye were evaluated microscopically. These nine sections (slides) were derived from three blocks/eye as follows. Three sagittal sections, oriented on plane, were trimmed from each eye. Each of these sections were processed to a separate paraffin-embedded cassette (i.e., block).*

  One of these blocks ("central") was a section through the (vertical) superior-to-inferior midline of the eye to include the optic nerve, lens, and most anterior curvature of the cornea. Taking this initial central section resulted in two approximately equal residual calottes on either side of the superior-to-inferior midline of the eye (i.e., nasal and temporal calottes). The other two blocks ("nasal" and "temporal") consisted of sections through the superior-to-inferior planes of these residual nasal and temporal calottes.

  From each of these three blocks (central, nasal, and temporal), three hematoxylin and eosin-stained microscopic slides were created. From each block, the first slide consisted of a section obtained after appropriate "facing" of the block, followed by two additional step sections (approximately 200 µm apart). Thus, the end result (nine sections per eye) consisted of three slides from each of the three blocks from each eye."

Peer Review: Yes. The peer review pathologist was Dr. (b) (4)

Histological Findings:

- Histopathological findings were limited to minimal changes in the treated (OD) eyes; with slightly more effects observed among females than males: conjunctival lymphoid hyperplasia, lymphocyte infiltration of the choroid and sclera, vitreal cells, and corneal vessels.
- No treatment-related systemic histopathology findings were apparent. Notably, no findings were observed in the spleen, adrenal glands, urethra or prostate.

o Two treated males exhibited minimal liver vacuolation. The relationship to treatment is unclear. In the absence of changes in liver weight or clinical chemistry parameters, this observation does not appear adverse.

Table 34: Selected male histopathology for the 6-month ivt monkey toxicology study (report # TDOC-0016684)

Parameter (D170 necropsy)	Group 1 0 (old formulation)	Group 2 0 (new formulation)	Group 3 1 mg/eye	Group 4 3 mg/eye	Group 5 6 mg/eye	Group 6 6 mg/eye (old formulation)
Right eye: minimal conjunctival lymphoid hyperplasia	0/3	0/3	0/3	0/3	0/3	1/3
Liver: minimal vacuolation	0/3	0/3	1/3	1/3	1/3	0/3

Table 35: Selected female histopathology for the 6-month ivt monkey toxicology study (report # TDOC-0016684)

Parameter (D170 necropsy)	Group 1 0 (old formulation)	Group 2 0 (new formulation)	Group 3 1 mg/eye	Group 4 3 mg/eye	Group 5 6 mg/eye	Group 6 6 mg/eye (old formulation)
Right eye: minimal conjunctival lymphoid hyperplasia	0/3	0/3	1/3	1/3	0/3	0/3
Right eye: choroid: minimal lymphocyte infiltrates	0/3	0/3	0/3	1/3	0/3	0/3
Right eye: sclera: minimal lymphocyte infiltrates	0/3	0/3	0/3	1/3	1/3	0/3
Right eye: minimal vitreal cells	0/3	0/3	0/3	0/3	1/3	0/3
Right eye: minimal corneal vessels	0/3	0/3	0/3	1/3	0/3	0/3

Toxicokinetics

- Blood was collected for TK after the first and last injections, at 6, 24, 48 and 168 hours post-dose. The detection method was a quantitative enzyme-linked immunosorbent assay (ELISA), with a lower limit of quantitation (LLOQ) of 2.00 ng/ml.

- For the new formulation (comparing groups 3, 4, and 5), dose-accumulation was apparent. Exposure was numerically lower for group 6 (6 mg/eye, old formulation) than for group 5 (6 mg/eye, new formulation).
- No apparent sex-difference for systemic TK (report page 954).

Table 36: Systemic TK for the 6-month ivt monkey toxicology study (report # TDOC-0016684)

Group	Formulation	Dose	Study day	Mean C_{max} (ng/ml)	Mean $AUC_{0\text{-}168h}$ (ng*h/ml)
3	New (50 µl/eye)	1 mg/eye	1	58.5 ± 21.2	2220 ± 1220
			141	172 ± 157	9770 ± 11,400
4	New (50 µl/eye)	3 mg/eye	1	162 ± 85.9	10,100 ± 5510
			141	273 ± 176	16,000 ± 12,800
5	New (50 µl/eye)	6 mg/eye	1	342 ± 158	15,500 ± 5240
			141	577 ± 413	30,300 ± 9580
6	Old (100 µl/eye)	6 mg/eye	1	313 ± 175	13,400 ± 7350
			141	352 ±128	15,400 ± 4030

Anti-drug antibody (ADA)

- The ADA analysis was confounded by pre-dose ADA against ranibizumab. After 6 months of treatment, the incidence of ADA increased. The ADA did not appear to affect TK.
- Anti-drug antibodies (ADA) were analyzed pre-dose (D0), after the first dose (D7), and after the last dose (D141 and D148) with a semiquantitative bridging ELISA method
    - Pre-dose ADA was detected in 2/12 controls, 3/6 low-dose, 3/6 mid-dose, and 3/12 high-dose monkeys.
    - At D141 and D148:
        - Controls: none of the control monkeys were positive (0/12).
        - Low-dose: one monkey was no longer positive, and one previously negative monkey was found to be positive for ADA
        - Mid-dose: the 3 previously positive remained positive; 2 previously negative exhibited ADA.
        - High-dose: the 3 previously positive remained positive, 6 more became positive (total of 8/12 positive at end of treatment).

Dosing Solution Analysis

Dose solution analysis was adequate. Characterization of the test articles were reported in Appendix C (report pages 100-143). The test articles were analyzed prior to the first dose, after the 4^{th} dose, and after the 6^{th} dose.

- All of the test articles were within 90% of nominal at all time points.
- Endotoxin was < 0.5 EU for all test articles.

Study title: AL-86810 (ESBA 1008, (b) (4) Drug Substance): A Three-Month Intermittent Dose (Q4Wx3) Intravitreal Toxicity Study with a Four Week Observation Period in Cynomolgus Monkeys	
Study no.:	• TDOC-0017689 • N-14-007
Study report location:	BLA module 4.2.3.2 Repeat-dose toxicity (\\cdsesub1\evsprod\bla761125\0001\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\pcs-rtdoc-0017689\pcs-rtdoc-0017689—pre-clinical-study-report.pdf)
Conducting laboratories and locations:	• In-life: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134 • Clinical pathology: (b) (4)
Report status and date:	Final, October 31, 2014
Date of study initiation:	April 28, 2014
Start of dosing:	May 6, 2014
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	Brolucizumab (AL-86810): • Vehicle lot # 14-68740-1 • AL-86810 120 mg/ml lot # 14-501477-1, purity ≥ 98%, endotoxin < 0.2 EU/ml • Vehicle and test articles were sterile (5 micron) filtered just prior to injection

Key Study Findings

- Groups of 3/sex cynomolgus monkeys were dosed by ivt injection OD monthly x 3 with 0 or 6 mg/eye of brolucizumab, and were necropsied 4 weeks following the last injection.
  - No ocular or systemic histopathology was performed. Therefore, the study design is not adequate to identify ocular or systemic NOAEL values.
  - No treatment-related effects were detected.
  - Spleen weight was increased for treated males compared to controls (but the magnitude was not adverse)
- Rationale for study:
  - The previous drug substance had been manufactured by (b) (4).
  - This study was conducted after the 6-month study (report # TDOC-0016684), to test the new drug substance manufactured by (b) (4). The (b) (4) drug substance was used for the Phase 3 trials, and (b) (4) is the current manufacturer for the to-be-marketed commercial drug substance.
- Formulation note: the vehicle formulation was (b) (4) sucrose, (b) (4) polysorbate 80, pH (b) (4). This formulation is close to, but not exactly the same as, the to-be-marketed clinical formulation.

- Note regarding change in protocol:
  - The original protocol specified that animals would be returned to colony at the completion of the study. In-life initiated May 6, 2014 and was completed July 30, 2014.
  - The protocol was modified on 5/30/2014, to add gross necropsy and organ weights. No explanation was provided in the study report, or in the Toxicology Written Summary (BLA module 2.6.6).

Methods	
Doses:	0 or 6 mg/eye
Frequency of dosing:	Once every 4 weeks x 3 (D1, D29, and D56, with necropsy 4 weeks later, on D86)
Route of administration:	OD ivt injection. • Under anesthesia, the globe was immobilized (forceps or a stay suture through the conjunctiva and Tenon's capsule) • "*A 30-gauge needle attached to a sterile syringe, containing the vehicle or test article will be passed through the sclera 3 to 5 mm posterior to the temporal limbus and angled posterior to avoid the lens. The target location of the injection is the inferior-temporal region.*" [i.e. same procedure as for report # TDOC-0016684]
Dose volume:	50 µl
Formulation/Vehicle:	(b) (4) sucrose, (b) (4) polysorbate 80, (b) (4) q.s. with water for injection
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3/sex/dose
Age at start of dosing:	Approximately 5 to 7 years
Weight at start of dosing:	3.5 to 7.2 kg
Satellite groups:	None
Deviation from study protocol:	Protocol deviations were record on report page 35; this reviewer concludes that they did not impact the outcome of the study, or interpretability of results

Observations and Results

Checks for Mortality and Morbidity; Clinical Signs; Body Weight, and Food Consumption

- Methods:

- Animals were checked twice daily (morning and afternoon) for morbidity and mortality. The afternoon check included an evaluation of food consumption and fecal/urine output.
- Cage-side observations were recorded weekly.
- Animals were removed from the cage for clinical observations once weekly.
- Body weights prior to each injection, 7 days after each injection, on D85
- Veterinary physical examinations were performed pre-dose and approximately 6 hours after each injection.

Ophthalmoscopy

- Methods:
  - Slit-lamp biomicroscopy to evaluate the conjunctiva, cornea, anterior chamber, light reflex, lens, and iris of both eyes; scored using the Hackett and McDonald scale. Assessed the day prior to each injection; the first, third, and seventh day following each injection; and on D85 (prior to necropsy)
  - Indirect ophthalmoscopy and IOP were measured on the day prior to each injection; the third day after each injection, and on D85 (prior to necropsy)
- Results:
  - No treatment-related ophthalmoscopic effects were apparent.
  - Transient procedure-related minimal ocular inflammation was observed (conjunctival congestion, aqueous flare, WBCs in the anterior chamber, cells in the vitreous)

ERG

- No treatment-related ERG effects were apparent.
- For the right eye (OD) only, photopic and scotopic ERG was performed prior to dosing, and "no more than" 14 days prior to D85. The ERG procedure was the same as for report # TDOC-0016684 (reviewed above). After dark adaptation, amplitude and peak time of the following waveforms were assessed:
  - Rod B wave (dark-adapted rod response)
  - Mixed A wave and mixed B wave (dark-adapted mixed rod-cone response)
  - Cone B wave (light-adapted cone-response)

ECG

- 4-lead ECGs were performed as part of the veterinary physical examination, under sedation: pre-dose and approximately 6 hours after each injection.
- ECGs were assessed qualitatively by Dr. (b) (4) (Cardiology), who concluded that "no qualitative changes" were detected for any animal. No data tabulation was provided.

- *Review note*: the timing of each endpoint did not cover the systemic T_{max} following intravitreal dosing. These data are of limited utility to support safety.

Hematology, Coagulation, and Clinical Chemistry

- o No treatment-related effects apparent for hematology, coagulation, or clinical chemistry.
- o Blood samples were collected pre-dose, and "no more than" 14 days prior to D85. Hematology samples were shipped "on cold packs" to (b) (4) and analyzed on the day of collection. Samples for serum chemistry and coagulation were frozen and shipped to (b) (4). Analysis was performed in compliance with GLP.
- o No urine samples were collected.

Gross Pathology

- Standard gross pathology was conducted. No lesions were reported for any animal.

Organ Weights

- At necropsy, weights were collected for a limited panel of organs: adrenals, brain, heart, kidneys, spleen, ovaries, and testes.
- Spleen weights were higher for treated males compared to controls: +9% for absolute spleen weight, and +16% for spleen:body weight.

Table 37: 6 mg/eye brolucizumab increased male spleen weight was increased in the 3-month (Q4Wx3) monkey ivt toxicology study (report # TDOC-0017689)

Groups	Parameters	Males	Females
Controls	D 85 body weight (g)	5925 ± 1490	3710 ± 644
	Spleen weight (g)	3.817 ± 0.211	2.920 ± 0.477
	Spleen:body wt	0.000644	0.000787
	Spleen:brain wt	5.150 ± 0.280	4.593 ± 0.640
6 mg/eye	D85 body weight (g)	5565 ± 1090 (94%)	3484 ± 157 (93%)
	Spleen weight (g)	4.177 ± 0.515 (109%)	2.853 ± 0.870 (97%)
	Spleen:body wt	0.000751 (116%)	0.000819 (104%)
	Spleen:brain wt	5.703 ± 0.683 (110%)	2.860 ± 1.561 (104%)

Results reported as mean ± standard deviation
Review note: D85 body weights documented on report pages 91 and 93. Spleen and spleen:brain weights documented on pages 107 and 111. Spleen:body weight calculated by this reviewer.

Histopathology

Tissues were preserved, but no histopathology was conducted.

Toxicokinetics

- Blood was collected for TK following the first and third injection at 0, 6, 24, 48 and 168 hours. Serum brolucizumab was measured "using a validated quantitative enzyme-linked immunosorbent assay (ELISA) analytical method with a lower limit of quantitation (LLOQ) of 2.00 ng/mL"
- No dose dose-accumulation is apparent.
- The authors note that a slight sex-difference is apparent for AUC (higher exposure in females).

Table 38: Serum TK for the 3-month (Q4Wx3) monkey ivt toxicology study (report # TDOC-0017689)

Day	Sex	C_{max} (ng/ml)	$AUC_{0\text{-}168\ h}$ (ng*h/ml)
1	Male	406 ± 290	14,400 ± 1870
	Female	308 ± 82.0	20,000 ± 321
56	Male	250 ± 52.2	15,500 ± 751
	Female	406 ± 39.2	20,600 ± 862

Results reported as mean ± standard deviation

ADA analysis

- The ADA results are not concerning, and do not appear to have affected TK or other study results.
- Blood was collected for ADA with the TK samples (i.e. D1 and D56), and also on D84.
- One control female (#1501) exhibited ADA pre-dose, D56 and D84.
- One treated female (#2502) exhibited ADA pre-dose, but not on D56 or D84.
- One treated male (# 2002) was negative for ADA pre-dose, but exhibited ADA on D56 and D84.

Dosing Solution Analysis

- Test article characterization was adequate. Potency was tested prior to the first and third doses, and was ≥ 97% of nominal.

7 Genetic Toxicology

Consistent with the advice provided in 2012 Guidance for Industry: ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals[32], no genotoxicity studies have been conducted with brolucizumab. In the BLA module 2.6.6 (Toxicology Written Summary), the Applicant notes that "Brolucizumab is composed entirely of naturally occurring amino acids and produced by recombinant DNA technology with no post synthesis chemical modification." P/T concurs that no genetic toxicology studies are needed to support the safety of brolucizumab.

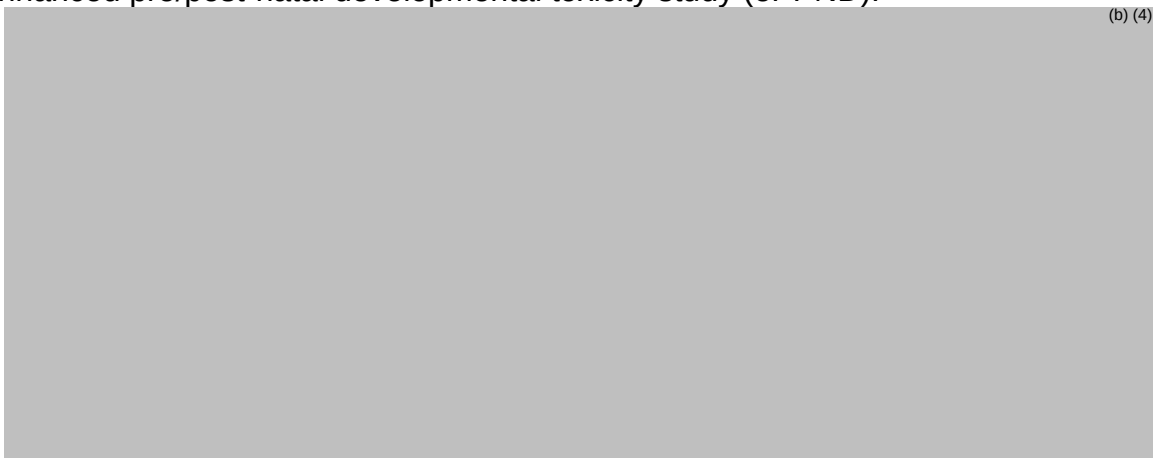
8 Carcinogenicity

- Consistent with the advice provided in 2012 Guidance for Industry: ICH S6(R1), no rodent carcinogenicity studies have been conducted with brolucizumab. P/T concurs that carcinogenicity studies are not needed to support the safety of intravitreal brolucizumab.
- The Applicant considered brolucizumab's anti-VEGF mechanism of action in their assessment of carcinogenic potential.

9 Reproductive and Developmental Toxicology

- No nonclinical reproductive and developmental toxicology (DART) studies were submitted to BLA 761125; none have been submitted to the parent IND.
- The Applicant considered the patient population, systemic exposure results, and the intended mechanism of action as part of the DART assessment.
- Under IND 112023, a type C pre-Phase 3 meeting[33] was held 1/15/2016 (minutes by Milstein, 2/12/2016). DART issues were discussed, including fertility and an enhanced pre/post-natal developmental toxicity study (ePPND).

(b) (4)



[32] Guidance accessible via: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s6r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals

[33] The briefing package was submitted to IND 112023 on 12/15/2015, and was accessed via: \\cdsesub1\evsprod\ind112023\0067\m1\us\meeting-clinical.pdf

(b) (4)

- No mention of a monkey fertility endpoints/study or ePPND study was identified in the BLA.
- The Applicant (b) (4)

(b) (4)

- P/T's previous recommendations (i.e. for the pre-Phase 3 meeting) were made based on the assumption that the monkey was the only pharmacologically relevant nonclinical model. In light of the PD study showing that the rat, mouse, dog, and pig may be pharmacologically relevant models, P/T plans to continue discussions on this topic under IND.

Lactation labeling issue

- For labeling section 8.2 Lactation, the Applicant proposed to recommend against breastfeeding for at least one month after stopping treatment with brolucizumab.
- During the filing review, P/T (McDougal, 3/26/2019, BLA 761125) drafted an Information Request (IR), which was conveyed (Semidey, 4/24/2019, BLA 761125) requesting a response by April 30, 2019: "The draft labeling has recommendations against breast feeding (section 8.2) and for women to use effective contraception (section 8.3) "for at least one month after the last dose". No explanation for this time point was identified. Provide scientific justification for the 1-month time point."

- The Applicant responded to the IR on 4/30/2019. Their response is acceptable.

(b) (4)

  - The 4-page "Responses to Questions Received 23 April 2019" was submitted under BLA module 1.11 (Information Amendment: Information Not Covered Under Modules 2 to 5)[35].
  - The justification of the 1-month recommendation is based on the systemic concentration detected at D28 following a single ivt dose in the Phase 2 clinical trial (report # RTH258-E003). The Applicant reports:
    - systemic elimination "declined in mono-exponential fashion" after ivt dosing, with an elimination half-life ($t_{1/2}$) of 4.4 days.
    - The serum lower limit of quantitation (LLOQ) was 0.500 ng/ml.
    - By D28, for the 6 mg brolucizumab dose group, brolucizumab was detectable in 13/25 patients (maximum concentration = 5.32 ng/ml; mean concentration = 0.540 ng/ml).
    - By D28, for the 3 mg brolucizumab dose group, brolucizumab concentrations were below the LLOQ.
  - The Applicant noted that brolucizumab does not contain an Fc moiety, and is not expected to bind to the neonatal Fc receptor (FcRN). Therefore, FcRN is not expected to contribute to placental transfer. P/T concurs.
- From a P/T perspective, the concentration of 5.3 ng/ml is expected to have significant pharmacological and toxicological activity (compare to the data tabulated in Table 41 below).
  - The safety of resuming breast-feeding after 1 month has not been established.
  - The duration of this recommendation should be re-considered, after more data are available.

11 Integrated Summary and Safety Evaluation

From a P/T perspective, ivt brolucizumab is safe for treatment of nAMD. Following distribution of brolucizumab from the eye into systemic circulation, systemic VEGF depletion is expected (with brolucizumab clearing from circulation prior to full recovery of circulating VEGF).

11.1 Ocular safety

- Early nonclinical studies performed under IND raised safety questions, which have been adequately addressed by improving the drug substance.
  - Pre-GMP lots of brolucizumab observed severe ocular inflammation in monkeys, which the Applicant attributes to impurities (e.g. endotoxin), and the early formulation.
  - Subsequently, GMP lots produced by (b) (4) continued to cause infrequent severe ocular inflammation in monkeys and rabbits.
- Ocular safety of the brolucizumab drug product is demonstrated by the GLP chronic monkey ivt toxicology study (report # TDOC-0016684). The high dose, 6

[35] Accessed via: \\cdsesub1\evsprod\bla761125\0006\m1\us\fda-response-clinical.pdf

mg/eye, administered once every 4 weeks x 6, was the ocular NOAEL. Ocular endpoints, which included photopic and scotopic ERG, were adequate to support safety.

- The proposed clinical dose is 6 mg/eye (b) (4) for the first 3 doses, (b) (4) P/T presumes bilateral clinical dosing.

Table 39: The nonclinical toxicology results demonstrate a 2-fold safety margin for ocular toxicity

Monkey ivt NOAEL (mg/eye basis)	6 mg/eye
Monkey ivt NOAEL (mg/ml basis, presuming a monkey vitreal volume of 2 ml)	3 mg/ml
Human ivt dose (mg/eye basis)	6 mg/eye
Human ivt dose (mg/ml basis, presuming an adult human vitreal volume of 4 ml)	1.5 mg/ml
Dose margin (on a mg/ml basis)	2-fold

11.2.1 Note regarding ERG

- The GLP toxicology studies included ERG. The descriptions in the study protocols and reports' methods sections were deficient.
- For brolucizumab, the available clinical safety data obviate the need to request additional information regarding the nonclinical study design (i.e. clearer ERG methodology).

11.2 Clinical PK: benchmark for P/T comparisons

- The Applicant's proposed labeling reports (section 12.3):
  - o
  - o (b) (4)
- As part of the justification for not conducting DART testing, the Applicant (BLA module 2.6.6 Toxicology Written Summary) cited clinical pharmacology results for the single ivt dose in the Phase 2 clinical trial (report # RTH258-E003)[36].

[36] For the clinical trial, the legacy clinical report was accessed via: \\cdsesub1\evsprod\bla761125\0001\m5\53-clin-stud-rep\533-rep-human-pk-stud\5333-intrin-factor-pk-stud-rep\rth258-e003\rth258-e003--legacy-clinical-study-report.pdf . The addendum of additional PK analyses was accessed via: \\cdsesub1\evsprod\bla761125\0001\m5\53-clin-stud-rep\533-rep-human-pk-stud\5333-intrin-factor-pk-stud-rep\rth258-e003\rth258a2201ad1--legacy-clinical-study-report.pdf

- The report states that patients were adults (≥ 50 years of age) with active choroidal neovascular (CNV) lesions secondary to AMD. Patients were dosed on D0, 25, and 56 with 3 mg (in 50 µl) or 6 mg (in 50 µl)
- From page 7 of the addendum:

Table 40: Clinical PK summary for the 6 mg/eye dose group (from report # RTH258E003)

RTH258E003 (CRTH258A2201)

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Descriptive Statistics for Brolucizumab Pharmacokinetic Parameters
(Pharmacokinetic Analysis Set)

Treatment: Brolucizumab 6mg

PK Parameters	n	Mean	SD	Geo.Mean	Min	Median	Max	Harmonic Mean(SD)
Day 1, C24hr (ng/mL)	25	65.7	107	35.9	8.02	31.3	548	
Day 57, C24hr (ng/mL)	25	45.0	59.9	27.9	4.87	25.1	299	
Cmax (ng/mL)	25	77.6	105	49.0	8.97	59.2	548	
AUC(0-tlast) (hr*ng/mL)	25	9160	12300	5320	910	4450	59400	
AUC(0-inf) (hr*ng/mL)	24	9770	12600	6000	1420	5580	60400	
Tmax (hr)	25	17.4	14.7	12.7	5.05	21.7	73.0	
t1/2(hr)*	24	123	50.8	113	38.0	122	245	103(52.5)

- n = number of subjects with Nonmissing values; SD = Standard deviation; Min = Minimum; Max = Maximum; Geo. Mean = Geometric mean.
- * Harmonic mean is only computed for t1/2. Jackknife estimate of the SD is presented.
- PK parameter estimates for AUC, Cmax, and half-life are related to the first injection.

- As benchmarks for systemic exposure margin calculations from 6 mg/eye, P/T will use:
  - **AUC = 9770 ng*h/ml**
  - **Cmax = 49.0 ng/ml** (taken from the draft labeling, and used instead of the 77.6 mg/ml value from the table above)
  - **Upper bound C_{max} = 548 ng/ml** (also from labeling)
  - **$t_{1/2}$ = 103 hours**

  Note: these values may underpredict risk, if patients receive bilateral ivt doses, or are dosed more frequently than once every 4 weeks.

11.3 PD results: comparison to clinical PK benchmark values

- The PD study reports presented K_D, EC_{50} and IC_{50} values in units of pM. This reviewer converted these values to ng/ml.

  - As described above: the molecular weight of brolucizumab is 26 kDa. The molecular weights reported in labeling are: bevacizumab= 149 kDa; aflibercept = 97 kDa; ranibizumab = 48 kDa.
  - For each protein, X value (in units of pM) multiplied by Y value (weight in kDa) = Z pg/ml. For conversion from pg/ml to ng/ml, multiply by 1000.
- As the table below illustrates, *the in vitro* PD data predict potent systemic activity following clinical ivt exposure. The limiting factors will be the total amount of VEGF available (versus the total amount of brolucizumab), and the rate at which systemic VEGF is synthesized to replenish circulating VEGF levels.

Table 41: I*n vitro* primary pharmacology results predict systemic activity, when compared to the clinical mean and upper-bound serum C_{max} values for 6 mg/eye

Report #	Parameter	Value	Value (ng/ml)	Exposure margin from the clinical benchmark C_{max} of 49.0 ng/ml	Exposure margin from the clinical PK upper bound C_{max} of 548 ng/ml
E1108S031.01	K_D (binding to huVEGF$_{165}$)	28.4 pM	0.7384	0.015 x	0.0013 x
	K_D (binding to glycosylated huVEGF$_{165}$)	21.6 pM	0.5616	0.011 x	0.0010 x
RD-2018-00365	K_D (binding to huVEGF$_{165}$ at 25^{o}C)	21 pM	0.546	0.011 x	0.0099 x
	K_D (binding to huVEGF$_{165}$ at 37^{o}C)	101 pM	2.626	0.053 x	0.0047 x
	IC_{50} (inhibition of VEGF-stimulated HREC proliferation)	54 pM	1.404	0.028 x	0.0025 x
	IC_{50} (inhibition of VEGF binding to VEGFR2 at 1 hr)	9.8 pM	0.2548	0.0052 x	0.00046 x
E1008S032.01	K_D (binding to huVEGF$_{165}$)	28.4 pM	0.7384	0.0150 x	0.0013 x
	K_D (binding to huVEGF$_{110}$)	25.2 pM	0.6552	0.0133 x	0.0011 x
	K_D (binding to huVEGF$_{121}$)	34.1 pM	0.8866	0.0189 x	0.0016 x

TDOC-0013037	IC_{50} (inhibition of VEGF-stimulated HREC migration)	0.093 pM	0.002418	0.000049	$4.41E^{-6}$
	IC_{50} (inhibition of VEGF-stimulated BREC proliferation)	0.77 pM	0.02002	0.000409	$3.65E^{-5}$

11.4 Systemic safety

- Review of the GLP ivt monkey toxicology studies noted observations of potential significance for systemic toxicity: increased spleen weight, lymphoid depletion, potential inguinal hernia, other skeletal muscle degeneration/necrosis, and broken teeth.
    - Of these, the incidence and severity of broken teeth, lymphoid changes, and muscle changes are not clearly treatment-related (see review above, and notes below).
    - The increases in spleen weight are clearly treatment-related. However, this reviewer considers a 2-fold increase in spleen weight to threshold for adverse nonclinical findings, based on risk of splenic rupture (personal communication, McDougal/Keegan and Lemry). Based on the magnitude of response observed with brolucizumab in monkeys, this reviewer concludes that the spleen weight is not adverse since it did not reach the 2X threshold. The lack of correlating histopathology and hematology supports this conclusion that the spleen weight is not adverse.

11.4.1 Inguinal hernia data – not clearly adverse; relationship to treatment unclear

- Evidence of inguinal hernia was observed in two male monkeys at 6 mg/eye after 6 months (report # TDOC-0016684).
    - The in-life finding was not well documented, and no follow-up by gross pathology or histopathology was documented to verify the initial observations.
    - No tissue cross-reactivity study was submitted for brolucizumab with human tissues. No nonclinical evaluation of systemic distribution study was conducted.
- The Q3Wx3 study noted minimal-to-moderate skeletal muscle degeneration/necrosis among treated animals. These findings are notable. However, they are insufficient to identify a mechanism of action.
- On July 31, 2019, an Information Request (IR) was conveyed to the Applicant: "*Regarding the 6-month intravitreal toxicology study in cynomolgus monkeys (report # TDOC_0016684), "possible" inguinal hernia was detected in two treated male monkeys on D161 by veterinary examination, prior to sacrifice on D170. Review of the study report (methods and protocol) did not find any description of the gross necropsy procedure. None of the documented gross pathology observations appear to correspond to the inguinal region.*

a. *Does the study laboratory's standard operating procedures (SOP) specify full, detailed gross necropsy which includes careful examination of the external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents for each animal?*
b. *Does the study laboratory's standard operating procedures (SOP) specify detailed exam at necropsy for any clinical observations/lesions noted in-life?*
c. *Were raw data recorded for gross necropsy of the inguinal area? If so, provide results for each animal.*
d. *Are relevant historical control data available for the laboratory (Alcon, Texas) for 2009-2014? If so, please provide this data."*

- The Applicant responded on 8/12/2019, confirming that standard full gross necropsy was performed, that would have detected abnormalities in the inguinal area.
  - The necropsy SOP was provided to the BLA; this reviewer concurs that the procedures are appropriate.
  - The SOP explicitly describes how to record gross lesions, including observed lesions for non-required tissues. For animals with no gross lesions, a summary entry of NGL (no gross lesion) is made. For animals with gross findings, only the findings are recorded (i.e. absence of findings in other tissues is inferred, but not captured for each tissue individually).
  - Both monkeys # 5001 and #6003 had no gross observations reported. Therefore, the Applicant concludes that the gross pathology of the inguinal area was normal for these monkeys, and this reviewer concurs.
  - Relevant historical control data were not available. The Applicant provided two literature references, which included descriptions of normal primate inguinal physiology, and descriptions of inguinal hernia.
- For this study, this reviewer concludes that the observations of inguinal hernia at veterinary examination, without apparent correlate at gross pathology, are not clearly adverse.
- Based on the lack of confirmatory gross pathology findings (e.g. strangulation), the relationship of the inguinal hernia to treatment is unclear.

1.14.2 Tooth data – no clear relationship to treatment

- The GLP Q3Wx3 monkey study (TDOC-0012707) observed broken teeth for one treated monkey. Because the published literature has reported an association between systemic VEGF depletion (by bevacizumab) with dental toxicity, , this reviewer checked the other GLP toxicology studies:
  - The GLP Q4Wx6 monkey study (TDOC-0016684) included gross pathology and histopathology for "teeth" – no concerns identified.
    - Not clear which teeth were checked. The protocol specifies that two sagittal sections were decalcified to allow for processing (page 970).
    - No gross pathology or histopathology findings were recorded for teeth.
    - One high-dose male (# 6002) had missing teeth noted pre-dose and subsequently as a clinical sign (i.e. not treatment-related)

- The GLP Q4Wx3 monkey study (DTOC_0017689):
  - One control male (#1001) was observed to have missing the first upper right incisor tooth at veterinary examination pre-dose (D-11) and on D56 (pages 217, 221)
  - Neither the gross pathology nor histopathology included tooth/teeth.

- Overall, this reviewer concludes that the monkey data do not support concern for potential tooth toxicity of brolucizumab exposure.

1.14.3 Exposure margin calculations

Table 42: Systemic exposure margins from the GLP ivt monkey toxicology studies to the clinical PK benchmark values (C_{max} and AUC)

Study #	Dose	Finding	Nonclinical C_{max}	Exposure margin for C_{max} (from 49.0 ng/mL)	Nonclinical AUC	Exposure margin for AUC (from 9770 ng*h/mL)
TDOC-0016684 (6-month ivt monkey study)	6 mg/eye	NOAEL	313 ng/ml	6.38 x	13,400 ng*h/ml	1.37 x
TDOC-0012707 (Q3Wx3 ivt monkey study)	6 mg/eye	NOAEL	286 ng/ml	5.83 x	20,700 ng*h/ml	2.11 x

Note: when TK values were available for multiple days (i.e. first and last dose), the approach of using lower nonclinical mean value was taken here (to protect patient safety)

11.4.4 Systemic safety deficiencies

- The nonclinical package is adequate to support safety for the proposed patient population (AMD). The anti-VEGF mechanism of action for brolucizumab is well-characterized and off-target toxicity is not expected. The patient population does not include children.
- It is important to note that the nonclinical safety package would have been inadequate to support a new molecular entity (NME) with a wholly novel mechanism of action.
  - None of the toxicology studies (GLP or non-GLP) included a full panel of systemic organ weights. None included urinalysis. These omissions would represent deficiencies for a less-well-characterized product.

- Per ICH S6(R1), "*when the NHP is the only relevant species, the potential for effects on male and female fertility can be assessed by evaluation of the reproductive tract (organ weights and histopathological evaluation) in repeat dose toxicity studies*". No treatment-related effects were detected by histology, and no changes in testes or ovary weights were noted. The lack of a full panel of reproductive organ weights (i.e. uterus, prostate, epididymis), and omission of other fertility endpoints from the general toxicity study design, are limitations.

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/s/

ANDREW J MCDOUGAL
08/14/2019 04:23:56 PM

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08/14/2019 05:36:19 PM